LATERALLY VERSUS MEDIALLY PROJECTING SPINOTHALAMIC NEURONS AND THEIR AXON COLLATERALS TO THE PERIAQUEDUCTAL GRAY AND MEDULLARY RETICULAR FORMATION IN THE RAT

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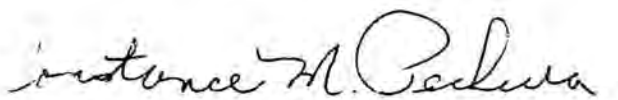

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ABSTRACT

Title of Dissertation: LATERALLY VERSUS MEDIALLY PROJECTING SPINOTHALAMIC NEURONS AND THEIR AXON COLLATERALS TO THE PERIAQUEDUCTAL GRAY AND MEDULLARY RETICULAR FORMATION IN THE RAT

Constance Mary Pechura, Doctor of Philosophy, 1987

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Fluorescent, retrograde tracing studies were undertaken to compare laterally (L-STT) versus medially (M-STT) projecting spinothalamic tract neurons in terms of their axon collaterals to the periaqueductal gray (PAG) or medullary reticular formation (MRF). The tracers used for these double-label experiments were fast blue or fluorogold in combination with rhodamine-labeled latex microspheres. Under general anesthesia, the tracers were injected into the lateral thalamus and MRF (N=6), lateral thalamus and PAG (N=5), medial thalamus and MRF (N=6), or medial thalamus and PAG (N=5). After 7 days, the animals were deeply anesthetized and perfused. The brains and spinal cords were removed and processed according to standard protocols. Locations of single- and double-labeled STT neurons were plotted for the spinal enlargements, mid-thoracic and upper cervical segments.

In all segments examined, single- and double-labeled L-STT and M-STT neurons were commonly observed in

contralateral laminae V-VIII and, at upper cervical levels, in ipsilateral laminae VII-VIII. The percentages of STT cells which were double-labeled (10-40%) in these areas were similar across the experimental conditions, but were generally highest in the upper cervical and lowest in mid-thoracic segments. The lateral spinal nucleus contained double-labeled STT neurons in all four experimental groups. More L-STT than M-STT neurons were observed in lamina I in the cervical enlargement some of these were double-labeled from the PAG, but not from the MRF. In the lumbar enlargement, many L-STT neurons, none of which were double-labeled, were seen in the ventromedial dorsal horn. Such populations of exclusively single-labeled L-STT neurons were also found at upper cervical levels in the internal basilar and lateral cervical nuclei.

These data suggest that many L-STT and M-STT neurons issue axon collaterals to the PAG or MRF. Further, L-STT neurons which project directly to the thalamus without axon branches are located in restricted spinal regions and may function in highly specific types of sensory processing.

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by

Constance Mary Pechura

Dissertation submitted to the Faculty of the Department of Anatomy
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for Gary,

Stephanie,

Mother,

and in memory of Dad

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INTRODUCTION

The mammalian spinothalamic tract (STT) represents an important pathway by which somatosensory experience is transmitted to certain thalamic nuclei and, through these nuclei, to the cortex. Because of the obvious clinical importance of neural pathways subserving somatosensation, especially pain, an enormous amount of research, beginning around 1890, has been devoted to the STT. As in all other areas of neuroscience research, investigation of the STT has produced bursts of new information as improved techniques became available. Nevertheless, some general concepts of the STT have emerged which seem to be enduring tenets. One of the most prominent ideas, based largely on the work of Mehler and his colleagues (1956, 1957, 1960, 1969), is that the STT is really two, mostly distinct, tracts. One tract courses medially through the brainstem to terminate in medial thalamic nuclei. The second tract separates from the other in the medulla and courses in a lateral position through the pons. This lateral tract terminates in the ventral posterolateral (VPL) nucleus of the thalamus. Many investigators, including Mehler, have suggested that these two components of the STT differ in function, collateral connections to other brainstem regions, and in their relative importance in phylogeny. It is important, therefore, to briefly trace the development of the two-component concept of the STT and to outline more recent research which in some respects amplifies the functional

significance of this concept, but in other respects contradicts it.

CLASSICAL ANATOMICAL STUDIES AND PHYLOGENETIC THEORIES

The two-component concept of the STT developed almost totally from data obtained using degeneration techniques to study axon pathways. These techniques, first developed by Marchi in 1885, allowed investigators to visualize distal, degenerating myelin sheaths following lesions of the spinal white matter. With this technique axons from the spinal cord were traced into the thalamic nuclei of the cat as early as 1890 by Edinger (see Mehler,1960). By 1910, spinal axons which ascended to terminate in the thalamus had been observed in a host of mammalian species including man. W. E. LeGros Clark (1936) was the first to define a topographically organized spinal projection to the monkey VPL. His experiments, also using the Marchi method, revealed terminations in the intralaminar thalamic nuclei and, for the first time, in the midbrain periaqueductal gray (PAG). Based on Marchi staining of human brains, Kuru (1949) described the course of degenerating ascending tracts resulting from anterolateral quadrant chordotomies. He described two tracts, a medial system which terminated mostly in the medullary reticular formation (MRF), which he called the "Tractus spino-thalamicus ventromedialis", and the peripheral or classical tract which he called the "Tractus spino-

thalamicus dorsolateralis". Kuru assigned the latter tract to function in the conduction of pain and temperature sensation to the nucleus ventralis a of von Monakow (corresponds to VPL and part of the ventral lateral nucleus, see Jones, 1985). Of the ventromedial tract, Kuru stated that it gradually diminished in size after supplying many collaterals to cranial motor nuclei and also terminated in nucleus ventralis a of von Monakow. He presumed that this tract conducted tactile and pressure sensation.

By 1949, the STT was believed to be comprised of two ascending tracts each of which terminated in VPL. Suggestions of STT axon collaterals to lower brainstem structures had been made (eg. Kuru, 1949). It is particularly important to note that the term "collateral" as used in the older literature did not always mean an axonal branch; rather, it often simply meant a group of axons which diverge from a common or continuing bundle of axons. The different usages of this term has contributed to confusion regarding the degree of actual branching of STT axons. Nevertheless by 1949, it seemed clear that the majority of spinal afferents terminated in the MRF because all of the studies to that date reported that degenerating axon bundles diminished in size, and terminations became more sparse rostral to the medulla. Thus, the MRF was considered to be a complicated way-station to the thalamus - the multisynaptic spino-reticulo-thalamic tract.

The possible functional importance of the MRF was

emphasized by Moruzzi and Magoun in a report published in the same year as Kuru's anatomical study. This landmark work (Moruzzi and Magoun,1949) showed that stimulation of parts of the MRF greatly increased cortical electrical activity as measured by the EEG. Between 1950 and 1958, the number of studies of spinal projections to the MRF increased greatly. The general pattern of degeneration in the MRF following spinal lesions was similar in cat, monkey and man (Brodal,1949; Morin, et al.,1951; Johnson,1954; Mehler et al.,1956; Bowsher,1957; Rossi and Brodal,1957; Nauta and Kuypers,1958). It was in such an environment that Mehler and his colleagues began their extensive comparative studies of the ascending spinal tracts of mammals. Mehler utilized the silver-impregnation method, developed by Nauta and Gygax in 1954, which allowed fairly precise definition of axon terminals. Such definition had not been possible with the Marchi stain. After preliminary reports (Mehler et al.,1956; Mehler,1957) Mehler et al. (1960) published a detailed report of the course, distribution, and terminations of the STT in monkey.

After lesioning the lateral or lateral and anterior white matter at cervical and thoracic levels of the spinal cord, Mehler and his coworkers mapped degenerating axons and terminals from the caudal medulla to the thalamus. They described areas of terminal degeneration in the MRF which they thought were possibly branches of ascending spinal fibers. This terminal degeneration was particularly dense

in the nucleus reticularis gigantocellularis. No distinct tracts were reported in the brainstem as far rostral as the superior olivary complex. At the level of the superior olives, the lateral component of the STT was reported to separate from a diffuse medial system. The lateral bundle contained larger diameter axons than the medial group. The two components rejoined in the rostral pons and remained "inextricably mixed" from that point to the most rostral extent of the tract. Terminal degeneration in the midbrain was especially dense in the PAG and in the deep layers of the superior colliculus. At the level of the medial geniculate nucleus it was observed that some fine fibers, "collaterals", left the tract and eventually entered the internal medullary lamina. These fibers terminated in the central lateral nucleus. The remaining fibers terminated in the VPL (also in the posterior nucleus and the magnocellular portion of the medial geniculate).

On the basis of these findings, the authors stated that the classical STT fibers, terminating in the VPL, represented a small portion of the total number of axons contained within the anterolateral funiculus. They further concluded that most of the terminations in the MRF were true spinoreticular fibers but that some of them may have been axon collaterals (branches) from the STT. Although the fibers to the PAG were characterized as "collaterals" in their description, the authors did not elaborate on this point in their discussion. Finally, it was concluded that

the STT fibers which terminated medially in the thalamus represented a phylogenetically old pathway; the classical STT terminated in the VPL and was a phylogenetically newer system. Both the MRF and PAG were implicated as relays for sensory information, especially pain, to reach the thalamus and higher brain centers.

Although published in abstract form and widely cited (Mehler,1957), it was not until 1969 that Mehler published a complete summary of his comparative studies (Mehler, 1969). The course and distribution of degenerating axons following anterolateral spinal cordotomy was described in monkey, chimpanzee, cat, rat, and opossum. The results were compared to and discussed in terms of the then current knowledge of such connections in non-mammalian species. The details conformed in most respects to the results in monkey discussed above. The only "striking" difference reported between primate and nonprimate species was that, in primates, the ascending fibers formed a compact bundle in the dorsolateral midbrain whereas these fibers were diffusely situated in rat and cat. Areas of terminal degeneration in the MRF, PAG, intralaminar and posterior nuclei of the thalamus, and in the VPL were generally homologous. However, there was a more complicated pattern of terminal endings in the primate VPL. In addition, it was suggested that the number of STT fibers projecting to the lateral thalamus was increased in primates. Previously, Mehler (1957) had reported that the laterally projecting

fibers represented 5% of the total number of ascending spinal fibers in the rat, 10% in cat, 20% in monkey, and 30% in chimpanzee.

In addition to describing his findings, Mehler devoted a large part of his 1969 paper to a detailed discussion of the various terminal regions and included a description of the phylogenetic theories of Herrick and Bishop (Herrick and Bishop,1958; Bishop,1959) regarding ascending pathways. Herrick and Bishop considered the medial, intralaminar thalamic nuclei of the mammal to correspond to the dorsal sensory area of a primitive thalamus in the salamander. Thus, the multisynaptic medial pathway from the spinal cord seen in salamander was present in mammals and represented by the spino-reticulo-thalamic tract. The ventrobasal thalamus or VPL was evolved in mammals due to the increased development of the cortex. Therefore, the spinal input to the VPL was regarded as uniquely mammalian. Bishop (1959) expanded this basic theory based on the fiber diameters of various ascending tracts and the sensory modalities carried by them. Basically, his theory stated that the more rostral a spinal ascending system terminated, the larger and more thickly myelinated were its axons and the more recent was its appearance in phylogeny. This, of course, implied that since touch and pressure were conducted to the spinal cord by large primary afferents, these modalities were transmitted via the larger axons of the classical STT.

Based on these theories, Bishop introduced the terms "paleospinothalamic tract" and "neospinothalamic tract" which subsequently came into common usage. In their strictest definition, neospinothalamic tract refers to those axons which terminate in the VPL and paleospinothalamic tract refers to direct or indirect projections which terminate in the intralaminar nuclei.

It is the present author's view that a number of misconceptions grew out of Mehler's work combined with the phylogenetic theories. The most important of these is the idea that axon collaterals to the MRF and PAG arose from the paleospinothalamic tract. Because the MRF and PAG were considered part of the phylogenetically old system, it was assumed that any collateral branches to them arose from axons destined to terminate in the medial thalamus. This conclusion was born despite the fact that Mehler (1960,1969) stated that at most medullary levels and at midbrain levels the two components of the STT are not separated and are indistinguishable from one another. It is also important to note that Mehler never unequivocally identified any true collateral branches of STT fibers. Finally, the phylogenetic theories were based on comparison of mammalian species to non-mammalian species; a much wider gap exists between these than between rat and primate. In fact, in his concluding remarks regarding the ascending tracts of mammals, Mehler (1969) stated, "... we, no doubt like others before us, have been more impressed with the similarities

that we have observed in the organization of the central nervous system than we have with the dissimilarities. To date, our search for clear-cut interspecies differences has revealed only one endpoint or regression in the spectrum of 'phyletically' constant spinal projections to the brainstem. This endpoint is represented by the apparent withdrawal of spino-olivary connections in chimpanzee and man...". The endpoint then refers to projections to the inferior olivary complex which has not been discussed here and does not refer to the STT.

PHYSIOLOGICAL STUDIES OF THE STT

Rarely is an enduring concept a result of one study however broad it may be; the two-component concept of the STT is no exception. Concurrent with Mehler's work, there were physiological data which supported a separation of the STT according to its medial versus lateral thalamic termination.

Melzack and Casey (1968) offered a unifying concept of pain processing which took into account these physiological studies along with known anatomical connections and clinical case studies. Since the VPL had been shown to receive a somatotopically organized afferent input from the spinal cord which carried tactile sensation with a high degree of place specificity, and since the VPL projected to the primary somatosensory cortex, Melzack and Casey proposed that the neospinothalamic tract was

responsible for the sensory-discriminative aspect of pain perception. The medial STT, with both direct projections and indirect ones through the MRF and PAG, was proposed to subserve the motivational and affective aspects of pain. This was based upon a lack of evidence of somatotopy or modality specificity in electrophysiological recordings from the intralaminar thalamus and because of the known limbic system connections of these regions.

At the time that Melzack and Casey formulated their model, electrophysiological investigations of the STT did not include precise characterization of the STT cells of origin. The studies typically involved extracellular recording of thalamic neurons and their responses to cutaneous stimulation. Later electrophysiological experiments had greater precision due to the use of antidromic activation to identify and characterize the response properties of the STT's cells of origin. By electrically stimulating the axon of a cell near its termination, one can evoke an action potential which is conducted from the terminal to the cell body (antidromically) where the potential can then be recorded by a nearby electrode. In this way, one can with reasonable certainty identify a spinal neuron as projecting to the region of stimulation. Further, by recording near that cell's soma, it is possible to characterize the cell's responses to cutaneous stimuli. Although the antidromic activation technique was applied quickly to the study of the

STT, it was first used to compare the response properties of medially versus laterally projecting STT neurons by Giesler and his coworkers (1981). Neurons antidromically activated from the VPL in monkey exhibited small, restricted receptive fields and most of them were of the wide-dynamic-range type which responded to innocuous cutaneous stimuli but discharged maximally in response to noxious stimuli like pinching. In contrast, STT neurons projecting to the medial thalamus were found to have large, often bilateral, receptive fields and most were high-threshold type neurons which respond only to noxious stimuli. In addition, it was reported that laterally projecting STT neurons had faster conduction velocities than medially projecting cells, implying that the latter type had smaller axons. On the basis of careful localization of the spinal electrode placements, these workers found that the majority of cells activated from the VPL were located in the dorsal horn whereas the medially projecting neurons tended to be in the intermediate gray zone. Thus, the results of this study generally supported the concept of a two-component STT system. However, the study also found that some STT neurons projected to both the VPL and to the medial thalamic nuclei via a branched axon. Interestingly, these neurons exhibited response properties typical of neurons projecting to VPL only. More surprising was the finding that some STT neurons could also be activated from the MRF with electrical stimulation parameters which indicated that these STT cells

had an axon collateral which terminated in the MRF. The response properties of these neurons were again typical of laterally projecting STT cells. These results seemed to contradict the idea that only the medially projecting STT neurons had axon collaterals to other brain regions. Yet, this was not an isolated finding. Price et al. (1978) had already reported that STT neurons in monkey could be antidromically activated from both the VPL and the PAG. Again, these neurons' response properties mirrored those of neurons projecting to the VPL alone.

FUNCTIONAL IMPLICATIONS OF STT COLLATERALS TO THE MRF AND PAG

By the middle 1970's, intriguing new functional roles were proposed for the MRF and PAG making their afferent input from the spinal cord an even more compelling area of research. It was discovered by Reynolds (1969), and expanded upon by Mayer and Liebeskind (1974), that electrical stimulation of the PAG and other medial brainstem regions elicited a powerful analgesia in animals; a phenomenon referred to as stimulation-produced analgesia. Research into this phenomenon mushroomed quickly and by 1978 Basbaum and Fields presented a unifying conceptual model of the mechanisms underlying descending modulation of spinal neuron activity. This model was updated in 1984 by the same authors.

It is now thought that stimulation of the PAG

activates neurons which project to the MRF, specifically the nucleus reticularis gigantocellularis, nucleus reticularis magnocellularis and the nucleus raphe magnus. Neurons in the raphe magnus receive convergent input from the PAG and other MRF regions and, via a pathway in the dorsolateral funiculus, project to the spinal dorsal horn. Spinal neurons responding to nociceptive or noxious stimuli are inhibited by this pathway (Basbaum and Fields, 1984). These authors suggested that spinal projections to the PAG and MRF may influence this descending system. If such an influence is also mediated by STT axon collaterals to these regions, then the functional implications of the two-component STT system become broader.

Since electrophysiological experiments have found differences in the response properties of medially versus laterally projecting STT fibers, it can be surmised that the nature or quality of the information reaching the PAG and MRF is heterogenous depending on whether the input is carried by a true spinomesencephalic or spinoreticular neuron or by an axon collateral of a medially versus laterally projecting STT neuron. Although little is now known regarding the normal physiologic functioning of the descending modulatory system, it will be important to a future understanding to know the anatomical connectivity of these regions in as much detail as possible.

RETROGRADE TRACING STUDIES OF ASCENDING SPINAL SYSTEMS

The advent of horseradish peroxidase (HRP) as a retrograde neuronal marker allowed for the mapping of the spinal neurons of origin of not only the STT but also of many ascending tracts including the spinoreticular, spinomesencephalic, and spinoannular (to the PAG) tracts. Giesler et al. (1979a) mapped STT neurons in rat which were labeled following injection of HRP into medial versus lateral thalamic structures. Exclusively laterally projecting neurons were found in a few spinal regions, such as the lateral cervical nucleus of the upper cervical spinal cord and in lamina I especially in the cervical enlargement. However, areas such as laminae V, VI, VII, and VIII contained both medially and laterally projecting STT neurons. The dorsal horn of the cervical and lumbar enlargements tended to contain more cells which projected to VPL while the intermediate gray zone in these segments contained more neurons which projected to the intralaminar nuclei. This latter finding was similar to their electrophysiological results in monkey (Giesler et al.,1981).

Comparison of these results with many later studies using single-label retrograde tracing methods in rat indicates that in the lateral, reticulated part of lamina V and in laminae VI, VII, and VIII there exist overlapping populations of neurons whose axons form the spinoreticular, spinomesencephalic/spinoannular and spinothalamic tracts

(Abols and Basbaum,1981; Chaouch et al.,1983; Granum,1986; Kemplay and Webster,1986; Liu,1983; Wiberg and Blomqvist,1984). However, none of these studies were able to determine which, if any, of these neurons belonged to more than one of these systems.

The use of multiple-label, fluorescent retrograde tracing techniques has made it possible to label neurons whose axons issue collateral branches. These methods take advantage of the different properties of various fluorescent tracers, such as color or cytoplasmic versus nuclear localization. Thus, two or more tracers can be distinguished from one another if both are present in a single neuron. These methods have been applied successfully to the study of ascending spinal systems. Kevetter and Willis (1983) used fluorescent tracers in combination with each other or with HRP to identify neurons which could be double-labeled following injections into the MRF and the thalamus of the rat. Double-labeled neurons were located in spinal laminae (V, VI, VII, VIII) which had been shown previously to contain STT and spinoreticular tract neurons. Following injection of tracers into the medial thalamus and the PAG, double-labeled neurons were observed in these same laminae of the cervical and lumbosacral spinal cord (Liu,1986). Finally, spinal neurons which project to the PAG and MRF via axon collaterals have been identified in these laminae of the lumbar and cervical enlargements and of the upper cervical spinal cord (Pechura and Liu,1986).

Unfortunately, neither of the two studies showing double-labeled STT neurons were able to address the lingering question of possible axon collaterals from laterally projecting STT neurons. In Kevetter and Willis' study (1983), the thalamic injections were large and spanned both medial and lateral thalamic nuclei. A more systematic approach was clearly necessary to the further study of the STT.

The present experiments utilize fluorescent, retrograde tracing methods in order to describe medially versus laterally projecting STT neurons in terms of their axon collaterals to the PAG and MRF.

MATERIALS AND METHODS

All experiments involved the injection of fluorescent tracers which are retrogradely transported from the axon terminals to their cell bodies. There were five experimental conditions. In the four principal experimental groups, double-label studies were done in which fluorescent tracers were injected into the lateral thalamus and MRF (L-STT/MRF), lateral thalamus and PAG (L-STT/PAG), medial thalamus and MRF (M-STT/MRF), or into the medial thalamus and PAG (M-STT/PAG). In an additional group of animals, triple-label studies were attempted and tracer injections were made into the thalamus, PAG, and MRF. Placement of the injections was based on the stereotaxic coordinates for the rat of Paxinos and Watson (1982). Table 1 summarizes the intended targets and the coordinates used in these studies.

The fluorescent tracers used in these studies were fast blue (FB), fluoro-gold (FG), rhodamine-labeled latex microspheres (RhS), and diamidino-yellow di-hydrochloride (DY). The properties of these tracers are presented in Table 2. For double-label experiments, the tracer combinations used were FB and RhS or FG and RhS. DY was only used in triple-label experiments in combination with FB and RhS. In order to assess whether FB, FG, or RhS is taken up by undamaged axons of passage, each of these tracers were injected into the corpus callosum according to a method described by Sawchenko and Swanson (1981). These injections were placed just dorsal to the ventral hippocampal

TABLE 1 STEREOTAXIC COORDINATES USED FOR TRACER INJECTIONS

Group Classification	Intended Target	Adjustments from interaural zero Anterior/Posterior	Lateral	Dorsal/Ventral
L-STT	Ventral Postero-lateral n. of thalamus	+6.0	+2.5	+3.8
M-STT	Central lateral n. of thalamus	+5.5	+0.5	+4.2
PAG	Lateral PAG, level of superior colliculus	+2.0	+0.6	+4.3
MRF	n. reticularis gigantocellularis, level of facial n.	-1.8	+0.5	+0.5
Corpus Callosum	corpus callosum, level of ventral hippocampal comm.	+7.2	+0.5	-2.5

*measured from dura mater

TABLE 2 PROPERTIES OF FLUORESCENT TRACERS

Tracer	Solution	Excitation Wavelength	Color	Location	Comments	Reference
FB	5%, aqueous	365nm 420 nm	blue green	cytoplasm cytoplasm	some tissue necrosis at injection sites.	Kuypers et al., 1980
FG	2%, aqueous	365nm 420nm	gold yellow	cytoplasm cytoplasm	tissue necrosis at the injection site, tendency to leak out of cells	Schmued and Fallon, 1986
RhS	aqueous suspension	550nm	red	cytoplasm	labeled neurons difficult to see at low magnification (1-25X), sphere size = .02-0.2µm	Katz et al., 1984
DY	2% in 0.5% DMSO and water	365nm 420nm	pale yellow yellow	nucleus nucleus	tissue necrosis at injection site, low solubility in water, water, labels fewer cells long distance	Keizer et al., 1983

commissure so that spread from the injection center would involve the commissure. Any uptake of tracer by these undamaged axons would result in the presence of labeled cells in the hippocampus.

Male, Sprague-Dawley rats (225-250 g) were anesthetized with 7% chloral hydrate (35 mg/100g, i.p.) and a local anesthetic (2% lidocaine hydrochloride) was applied topically into the external auditory meatus and injected (s.c.) into the incision area. The animals were then placed into a Kopf stereotaxic apparatus. The skulls were exposed and burr holes were made in the crania with a dental drill to allow passage of glass micropipettes containing tracer solutions.

The injection system consisted of a one microliter glass syringe which had a side port at the base. A mineral oil-filled syringe was attached to this side port. Non-expandable Teflon tubing connected the tip of the syringe needle and a glass micropipette (25-75μm inner tip diameter). The entire system was filled with oil via the side port. Prior to injection, 1 μl of oil was first extruded from the micropipette by depressing the plunger of the syringe, the tracer substance was then drawn into the micropipette. After placement of the pipette into the target region, approximately 200 nl of tracer was injected by depressing the plunger of the microliter syringe. Injection volumes for the corpus callosum injections were 75-100 nl. In all cases, the pipettes were left in place

for 5 min following injection in order to minimize diffusion of tracers into the pipette track.

After the pipettes were withdrawn, the burr holes were packed with Gelfoam and the incision was sutured. Upon awaking from the anesthetic, the animals were returned to the University's animal care facility. They were housed individually, given free access to food pellets and water, and maintained on a 12 h light/dark cycle.

On the 7th post-surgery day (5th post-surgery day for corpus callosum cases), the animals were deeply anesthetized with 7% chloral hydrate (40 mg/100g) and perfused transcardially with 0.9% saline followed by 10% formalin. The brains and spinal cords were removed and stored in cold 10% formalin. Before sectioning, all tissue was cryoprotected by immersion in a solution of 30% sucrose in 0.1M phosphate buffer for 24 h.

Serial sections (40μm) were made through the injection sites on a freezing microtome and alternate sections were mounted onto gelatinized slides. In the corpus callosum injection groups, the sections extended past the injection site caudally to include the hippocampus. In all the rest of the cases, additional sections were taken (non-serial, 40μm) through the caudal medulla for assessment of labeling in the dorsal column nuclei and spinal trigeminal nucleus.

Initial assessment of the injection sites was done by microscopic examination using a Zeiss photomicroscope

equipped for epifluorescent microscopy. The nuclei which contained tracers were noted as well as the general extent of the injections. If this initial evaluation determined that the tracers were contained in the intended targets, then further analysis of the injection sites was undertaken.

In order to more rigorously assess the injection variablity between individual animals, measurements to determine the volume (mm^3) of tissue affected by tracer injections were made for each injection site. To accomplish this, slides containing the serial sections through the injection sites were placed on a microprojector and alternate sections were traced onto paper. The resulting drawings of the sections were 13.75X the area of the actual sections and each drawing represented a 160µm section thickness since every fourth, 40µm section was drawn. Depending on the specific tracer injected, each injection site exhibited from two to three different zones which varied according to the tracer used.

A Hewlett Packard 9874-A digitizer was used to measure the volumes from the drawings and the calculations were done by a Hewlett Packard 9835-A computer. A computer program was adapted for this purpose which took into account the magnification factor of the drawings and the section thickness to calculate the volume of tissue affected by each injection zone. This program was kindly provided by Dr. Kathryn Lynch and adapted by Mr. Michael Singer.

Sections through the dorsal column nuclei were

examined with epifluorescent microscopy. The number of cells labeled from the thalamus in 3-5 sections was counted to assess whether the injection involved the VPL. The dorsal column nuclei project heavily to this region and do not project heavily to the intralaminar thalamic nuclei. Cells in the dorsal column nuclei labeled from the PAG or MRF injection were also counted to determine whether these injections had spread into the medial lemniscus. A qualitative estimate of labeling was made by examination of the spinal trigeminal nucleus to determine whether the thalamic injections involved the ventral posteromedial nucleus of the thalamus, a region adjacent to VPL which receives massive input from the spinal trigeminal complex. Finally, since reciprocal connections exist between the VPL and the primary somatosensory cortex, the density of cells labeled from the thalamic injections was also estimated in the primary somatosensory cortex.

All of this information was considered in the decision to accept or reject a particular case. In general, cases were rejected if: 1) no tracer was found in one or both of the intended injection sites, 2) a lateral thalamic injection extended into the intralaminar nuclei or vice versa, 3) significant labeling occurred in the dorsal column nuclei, spinal trigeminal nucleus, and primary somatosensory cortex following medial thalamic injections, 4) a great deal of spread occurred from a PAG or MRF injection site, and/or 5) many cells were labeled in the dorsal column nuclei from

the PAG or MRF injection.

The spinal cords of accepted cases were blocked into upper cervical, cervical enlargement (middle portion), mid-thoracic, and lumbar enlargement (middle portion) pieces. Each of these was cut on the freezing microtome (25μm) and alternate sections were mounted onto gelatinized slides. The tissue blocks taken were larger than needed so that the resulting sections could be matched across cases according to the characteristic shape of the spinal gray matter for each level. These sections were also analyzed using epifluorescent microscopy. Cross-sectional tracings were made of a few sections at each of the four spinal levels with the use of a microprojector. Locations of single- and double-labeled neurons were recorded on these drawings. For each of the enlargements and mid-thoracic segments, 30 sections were analyzed for the location and number of cells single-labeled from the PAG or MRF. A total of 50 sections was examined for single- and double-labeled L-STT or M-STT neurons. In the upper cervical segments, 15 sections were analyzed for single-labeled cells projecting to the PAG or MRF and 30 sections were analyzed for single- and double-labeled STT neurons. In the triple-label experimental group, only single-, double-, or triple-labeled STT neurons were recorded systematically in 15 sections from the upper cervical and in 50 sections from each of the remaining spinal segments.

RESULTS

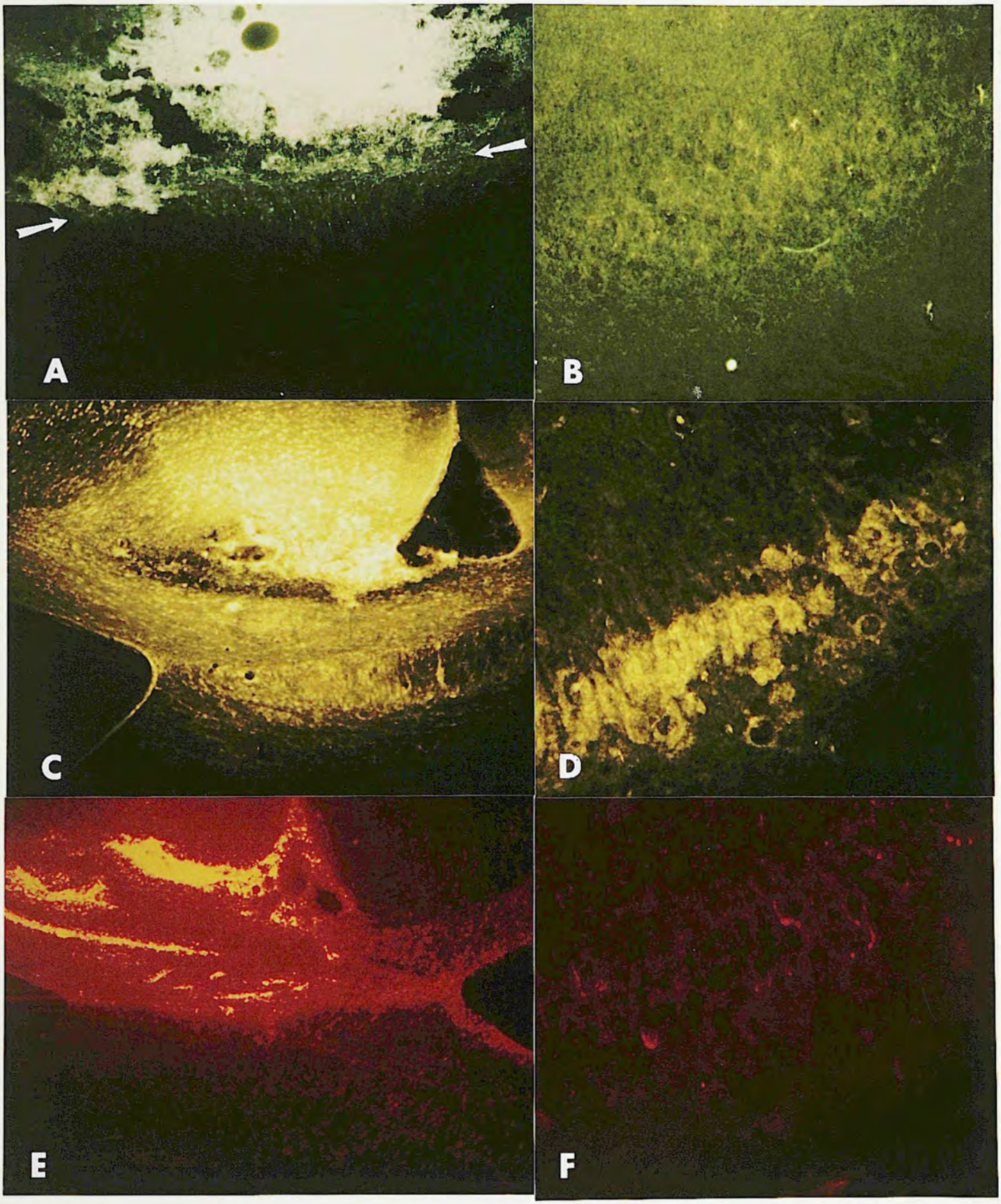
CORPUS CALLOSUM GROUPS

The issue of uptake by undamaged axons of passage is a critical one in double-label studies of the STT. There are STT axons passing in close proximity to the MRF and PAG injection sites (Mehler et al.,1960; Mehler,1969). Uptake of tracer by these axons could seriously confound the interpretation of double-label studies. Therefore, FB, FG, and RhS were tested to determine whether the tracers are taken up by undamaged axons of passage. Cases were accepted if: the center of the injection was in the corpus callosum or just dorsal to it; there was diffusion of tracer into the ventral hippocampal commissure; and there was no apparent damage from the pipette in the ventral hippocampal commissure. Figure 1 shows the injection sites of a case from each group and the resulting presence or absence of labeling in the hippocampus. Only FG exhibited significant uptake by undamaged axons of passage (Fig 1,C and D). FB-labeled cells in the hippocampus were present only if the commissure had been damaged; however, it was difficult to get enough diffusion of FB from the injection center to be certain that FB is not taken up by undamaged axons (Fig 1,A and B). True blue, a tracer similar to FB, has been shown to readily label hippocampal neurons under the same experimental conditions (Sawchenko and Swanson,1981). RhS was clearly not taken up by axons in the commissure (Fig 1,E

FIGURE 1

Photomicrographs of tracer injection sites in the corpus callosum and the resulting cell labeling in the hippocampus. Injections of FB (A), FG (C), and RhS (E) are shown at 37X. Arrows in A are at the boundary between the corpus callosum and ventral hippocampal commissure. Diffusion of FB (A) in case 809 into the commissure did not result in cell labeling in the hippocampus (B, 234X). In contrast, the injection in case 806 of FG (C) resulted in significant cell labeling (D, 234X). Diffusion of RhS in case 801 into the commissure is more apparent in color photographs due to the difficulty in obtaining clear photographs under 550 nm wavelength light. The diffusion present in this case did not result in labeled neurons in the hippocampus (F, 234X).

FIGURE 1



and F).

Certain specific properties of these tracers may account for the differences in uptake by undamaged axons. RhS is an aqueous suspension of labeled microspheres which are .02 to .2μm in diameter. Thus, the spheres are large enough to prevent passive diffusion across intact cell membranes and active uptake is unlikely to occur in passing axons. Additionally, it has been shown that RhS-filled neurons exhibit normal physiological responses, demonstrating that RhS has no toxic effects (Katz et al.,1984). In contrast, FB and especially FG seem to be somewhat toxic since there is usually tissue necrosis at injection sites of these tracers. Also, FG and FB may cross intact cell membranes as evidenced by my observation that FB- and FG-filled neurons sometimes have labeled glial cells in close apposition, indicating leakage of these tracers from the labeled neurons. It may be that undamaged axons in the injection sites of FB and FG become damaged with prolonged exposure to these tracers, allowing tracer uptake to occur.

Based on these different properties and the results of the corpus callosum injections, RhS was the tracer chosen for all of the PAG and MRF injections. Since the thalamus was considered the most rostral projection site for spinal neurons (Mehler,1969), axons of passage in the thalamus were not a critical problem in these studies. Therefore FB, and in a few cases FG, was chosen for thalamic injections.

DOUBLE-LABEL STUDIES: INJECTION SITES

Thalamus

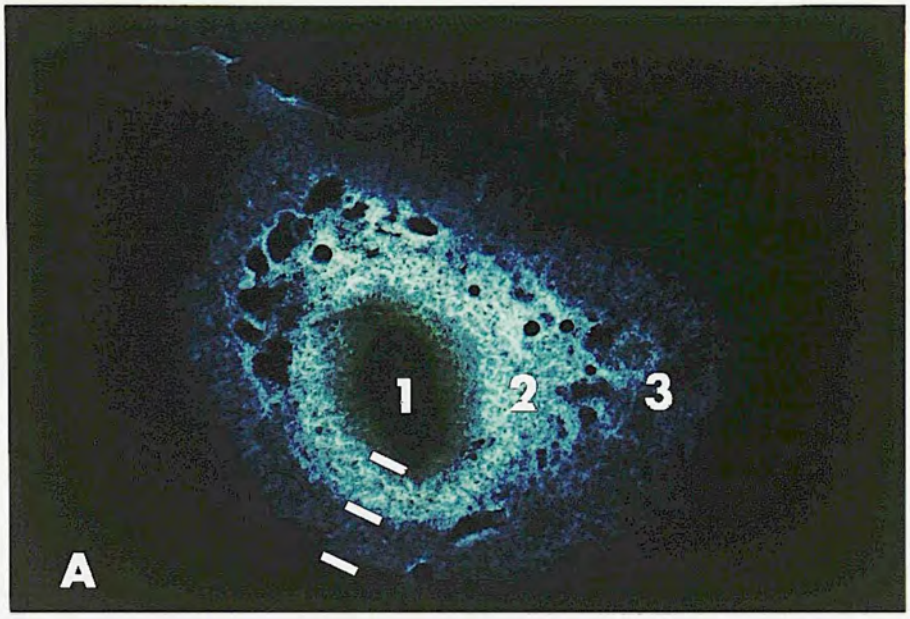
The FB and FG injection sites in the thalamus exhibited three zones which were most distinct with FB injections (Fig 2). The central zone of the injection site contained most of the tracer substance which had been expelled from the pipette. An area of tracer diffusion surrounded this central zone. Finally, a third, peripheral zone was observed in which there was intense cell labeling. Uptake of tracer by axon terminals, damaged axons, and,possibly, undamaged axons is considered to occur from the first two zones only (Kuypers et al.,1980; Schmued and Fallon,1986).

The thalamic nuclei affected by the tracer injections were determined using the nuclear divisions of Paxinos and Watson (1982) for the rat (Fig 3). In general, L-STT (N=11) injections were centered in the VPL (Fig 3 B,C&D). In more rostral L-STT injections, the area of diffusion and/or part of the injection center was observed to involve the ventral lateral and reticular thalamic nuclei (Fig 3,A&B). Injections placed more caudally often involved the ventral posteromedial and posterior thalamic nuclei (Fig 3 C&D). In contrast, M-STT (N=11) injections were centered in the central lateral nucleus and most often involved the mediodorsal, paracentral and centromedian thalamic nuclei (Fig 3 B,C&D). In a few cases, a small part of the

FIGURE 2

Photomicrographs of a FB injection (A, 37X) and a FG injection (B, 37X). In A, FB was injected into the medial thalamus of case 139. The photomicrograph was taken under 365 nm wavelength light. Three zones are seen in this site, indicated by numbers and bars. The center zone (1) is quite dark and is surrounded by a zone of tracer diffusion (2). The light outer zone (3) is a region where dense cellular labeling was observed. The first two zones are considered to be effective uptake zones for this tracer. In contrast, the FG (B) injection from case 315 exhibits indistinct zone boundaries, especially those between the central and diffusion zones. The outermost (arrows) zone of the FG injection also contains dense cellular labeling.

FIGURE 2



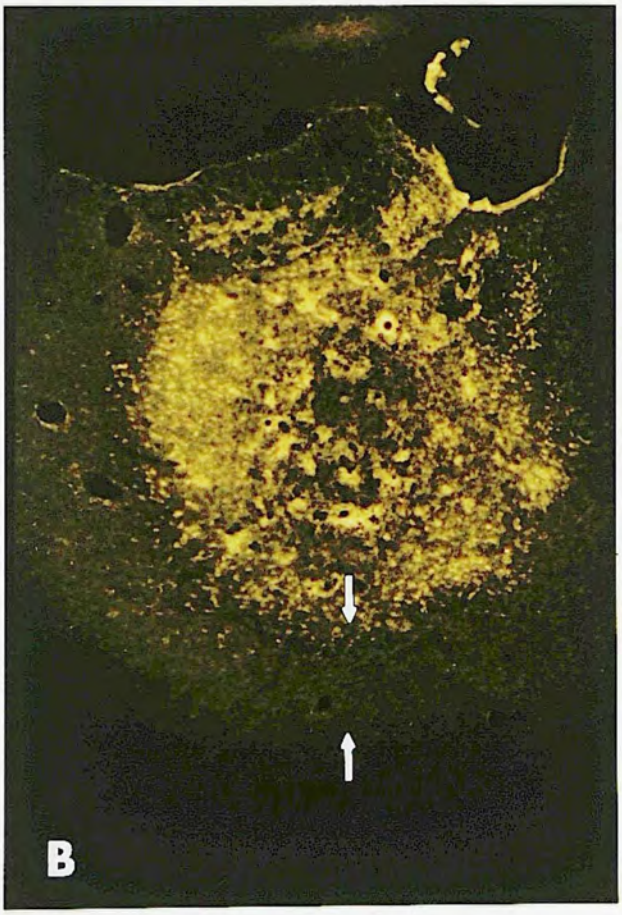
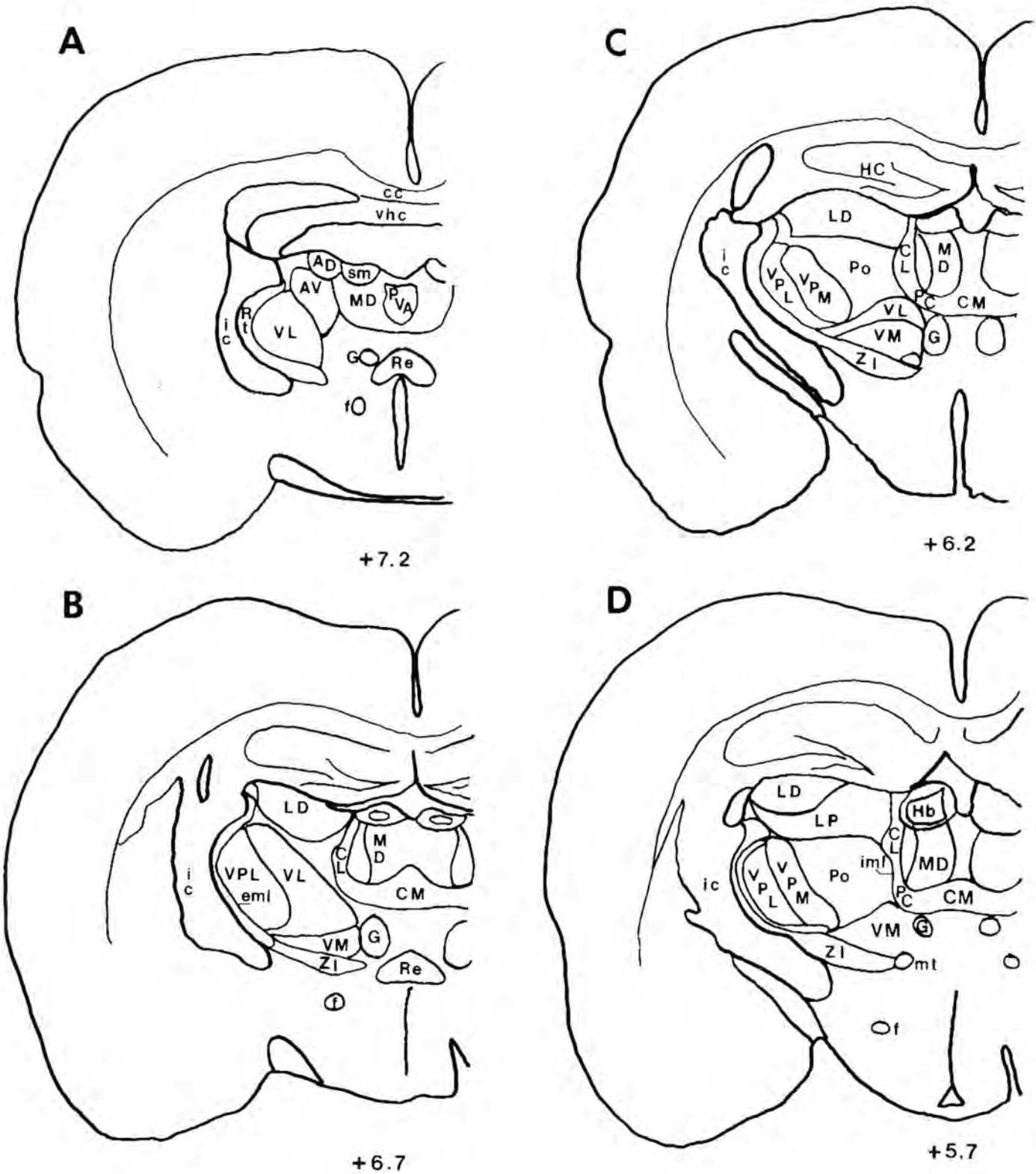


FIGURE 3

Line drawings from transverse sections illustrating the thalamic nuclear divisions used in this study. Nuclear boundaries are taken from Paxinos and Watson (1982). These drawings illustrate four rostro-caudal (A-D) levels of the thalamus. Anterior coordinates from interaural zero are noted beneath each drawing. See page 215 for list of abbreviations.

FIGURE 3


A
cc
vhc
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AV
MD
PVA
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Rt
VL
G
Re
fO
+7.2
B
LD
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D
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VPL
VL
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VM
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f
+6.7
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HC
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VPL
VPM
Po
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PC
CM
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D
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ic
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mt
f
+5.7

injection involved the most medial portions of either the ventral lateral nucleus or the posterior nucleus. In these studies, the internal medullary lamina limited most tracer diffusion in M-STT injections from the central lateral nucleus into either the ventral lateral or posterior nuclei. Diffusion was also not often seen to cross the external medullary lamina in L-STT injections. Finally, neither the medial or lateral thalamic injections involved the gelatinosus nucleus of the thalamus, a region which is also called nucleus submedius.

The volume of tissue affected by each injection is presented in Table 3. The central zones of L-STT injections centers involved an average of 1.480 $\pm$ 0.195 (S.E.M.) mm^3 of tissue. An average of 3.872 $\pm$ 0.532mm^3 of tissue was contained in the total effective uptake zone, that is, the central zone and diffusion zone combined. Generally, M-STT injections were smaller than L-STT injections. An average of 0.913 $\pm$ 0.132mm^3 of tissue was affected by the centers of M-STT injections. The total effective uptake zones averaged 2.799 $\pm$ 0.366mm^3 in M-STT cases.

The observations of neurons labeled from L-STT and M-STT injections in the dorsal column nuclei, spinal trigeminal complex, and primary somatosensory cortex are summarized in Table 4. L-STT injections resulted in significant labeling of cells in these regions, whereas M-STT injections did not.

Based on these data, it is most conservative to

TABLE 3

VOLUME OF TISSUE AFFECTED BY TRACER INJECTIONS INTO THE THALAMUS (mm^3)

Case	Tracer	Injection center	Center + Diffuse Zone
L-STT/MRF			
124	FB	2.823	6.895
130	FB	0.870	2.081
131	FB	1.167	2.350
132	FB	1.437	3.257
133	FB	1.033	2.963
136*	FG	0.844	2.703
L-STT/PAG			
501	FB	1.138	3.327
502	FB	1.877	4.436
503	FG	1.850	5.679
504*	FB	0.826	1.968
505	FB	2.415	6.935
M-STT/MRF			
126	FB	0.704	3.483
128	FB	1.233	2.794
129*	FB	0.673	2.653
139	FB	0.521	1.661
140	FB	0.930	2.632
142	FB	0.249	1.124
M-STT/PAG			
301	FB	2.043	6.019
303*	FB	0.830	3.257
312	FB	1.024	2.536
314	FB	0.913	1.856
315	FG	0.925	2.777

*Case is individually illustrated

TABLE 4

SUMMARY OF DATA FROM AREAS USED AS CHECKPOINTS FOR THALAMIC INJECTIONS

Case	N. Gracilis	N. Cuneatus	Spinal Trigeminal N.	Sensory Cortex
L-STT/MRF				
124	++++	++++	***	***
130	++++	+	*	**
131	++++	++++	***	***
132	++	++	***	***
133	++++	+++	***	***
136 x	++++	++++	***	**
L-STT/PAG				
501	+	++	*	**
502	++	+	***	***
503	++	++	***	***
504 x	++++	++++	***	***
505	++++	++++	***	***
M-STT/MRF				
126	+	+	**	**
128	-	+	-	*
129 x	-	-	-	*
139	-	+	-	-
140	+	+	*	*
142	-	-	*	**
M-STT/PAG				
301	-	+	-	*
303 x	-	-	-	*
312	+	-	-	*
314	+	-	*	*
315	+	+	-	*

x Case is individually illustrated

\+ 3-10 cells labeled
++ 11-20 cells labeled
+++ 21-30 cells labeled
++++ more than 31 cells labeled in one 40 µm section. Data pooled from 3-5 sections.

* Few cells labeled
** Low to moderate labeling
*** many cells labeled in one 40 µm section

consider that L-STT cases resulted in labeling of spinal neurons which project to VPL, ventral lateral, and/or posterior thalamic nuclei since these regions were affected by tracer injections and they have been shown to receive spinal afferents (Mehler et al.,1960; Mehler,1969). On the other hand, M-STT cases should be considered to result in the labeling of spinal neurons which project to the intralaminar thalamic nuclei, especially the central lateral nucleus, as there was little diffusion of tracer outside this region.

PAG and MRF

The RhS injection sites in the PAG and MRF exhibited only two zones. The center of the injection contained the RhS expelled from the pipette and the second zone contained an area of diffusion from the center (Fig 4). Based on the data from the corpus callosum injections, it can be assumed that the diffusion zone did not label any undamaged axons of passage. However, whether or not the total effective uptake zone for axon terminals includes the diffusion area is not clear for RhS.

The boundaries of the PAG for mapping of the injection sites were defined according to Paxinos and Watson (1982) and did not include any subnuclei. Specific MRF nuclei were defined according to the divisions described by Newman (1985). These MRF divisions are depicted in Figure 5. Injections into the MRF (N=12) were centered in the

FIGURE 4

Photomicrograph (19X) illustrating a RhS injection site taken under 550 nm wavelength light. The bright areas are the injection centers which are surrounded by the zone of diffusion. Unlike FB and FG, RhS injections do not result in an obvious third zone of dense cellular labeling. In general, photography at this magnification and wavelength overexposes the diffusion zone, it therefore appears more dense than it actually is. In addition, the details of the surrounding brain tissue are lost when photographing under this wavelength of light. However, the use of other wavelengths to enhance the surrounding detail results in photographs with severely underexposed diffusion zones.

FIGURE 4

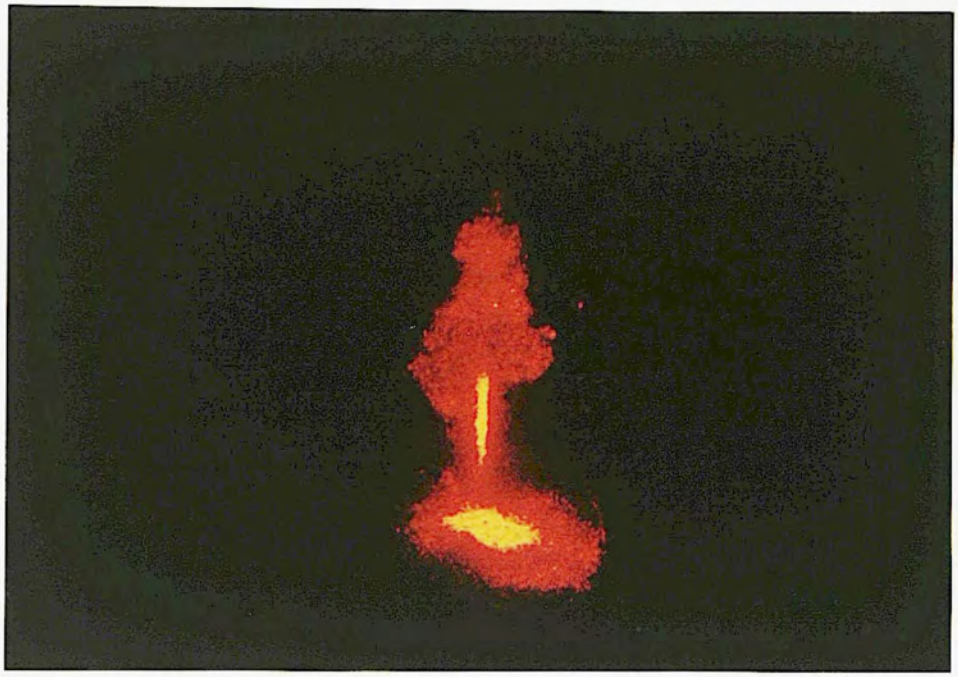
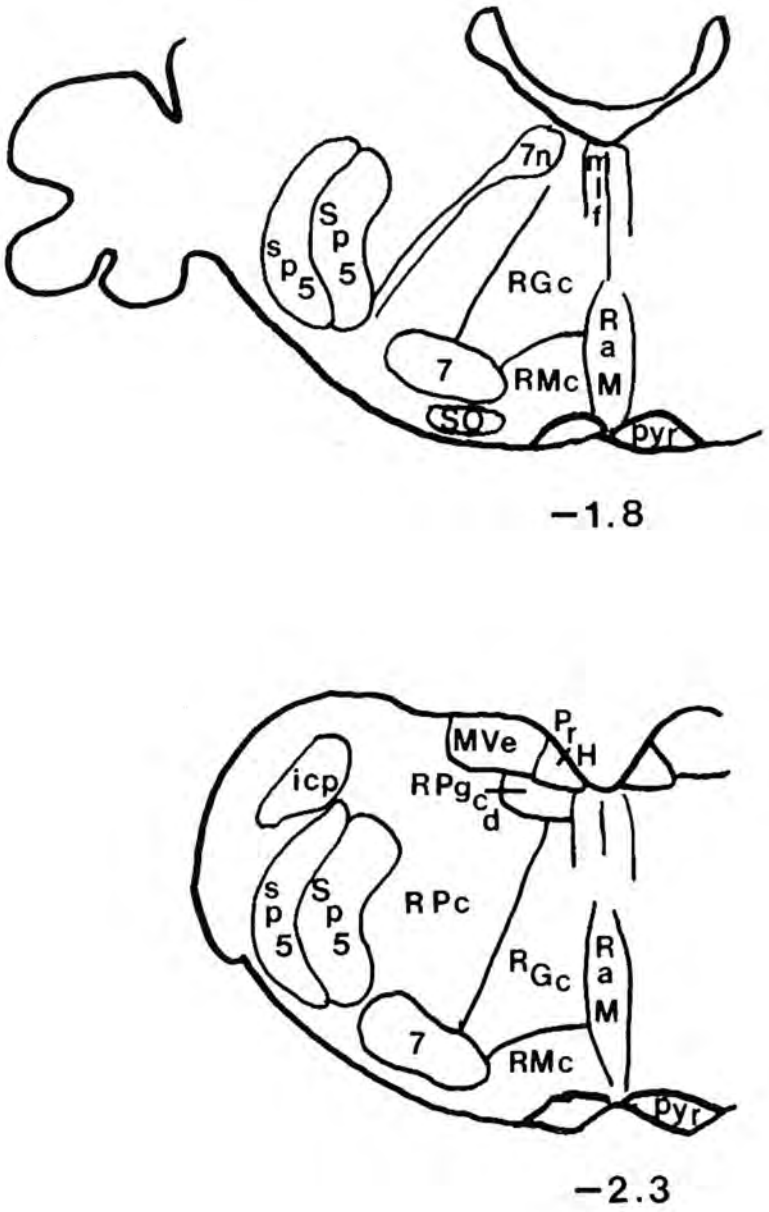


FIGURE 5

Illustration of the MRF nuclear delineations used in this study, which were taken from Newman (1985). Other nuclear boundaries are from Paxinos and Watson (1982). See page 215 for list of abbreviations.

FIGURE 5


7n
mlf
Sp5
spV
RGc
Ra
M
7
RMc
SO
pyr
−1.8
MVe
Pr
H
icp
RPgcd
spV
Sp5
RPc
RGc
Ra
M
7
RMc
pyr
−2.3

nucleus reticularis gigantocellularis at the level of the facial nucleus. In some cases the injections impinged upon the medial portion of nucleus reticularis parvocellularis laterally or the nucleus reticularis magnocellularis ventrally. No diffusion of tracer into the pipette tracks through the cerebellum was observed.

PAG injections (N=10) were centered in the lateral PAG at the level of the superior colliculus. There was almost always a small amount of tracer which diffused or was dragged by the pipette tip into the superior colliculus. Often, RhS was observed lining the inside of blood vessels. At the level of the PAG, circumferential arteries and veins penetrate into the brain parenchyma in such a way that, in transverse sections of the midbrain, these blood vessels are cut tangentially. Thus, they seem to radiate from the PAG to the surface of the brain like the spokes of a wheel. RhS was sometimes seen inside these vessels, giving the misleading appearance of a great deal of tracer diffusion outside the PAG. It is unlikely that this problem resulted in labeling of passing axons since RhS was not observed to be leaking out of these blood vessels and since the axons through these areas were almost certainly not damaged. Nevertheless, cases in which this problem was extensive were rejected. Finally, in the MRF injections, a blood vessel cut in cross-section was also sometimes seen packed with RhS. These did not give the appearance of injection spread due to the plane of section. Little or no

leaking was observed from these vessels.

The volume of tissue affected by RhS injections into the PAG and MRF are reported in Table 5. The injection centers were smaller in the PAG ($\bar{X}$=0.164 $\pm$ 0.031mm^3) than in the MRF ($\bar{X}$=0.248 $\pm$ 0.041mm^3). However, there were comparable volumes of tissue affected by the combined central and diffusion zones ($\bar{X}$=1.238 $\pm$ 0.255mm^3 for the MRF versus $\bar{X}$=1.144 $\pm$ 0.273mm^3 for the PAG).

In the cases accepted, no significant cell labeling in the dorsal column nuclei followed tracer injections into the PAG or MRF (Table 6).

Injection sites of four individual cases

One specific case has been chosen from each double-label group for individual illustration; these are cases 136 (Fig 6), 504 (Fig 7), 129 (Fig 8), and 303 (Fig 9). These cases were chosen on the basis of their being generally representative of their groups in terms of injection sites and spinal labeling.

In Figure 6 the injection sites of a L-STT/MRF case (136) are depicted in line drawings of sections through three, rostral to caudal levels of the injection sites. In this case, the FG injection was centered in the VPL on its border with the ventral lateral nucleus. The core or central zone extended from the rostral pole of VPL to and including the rostral part of the posterior nucleus. The diffusion zone impinged upon the VPL, ventral lateral,

TABLE 5

VOLUME OF TISSUE AFFECTED BY TRACER INJECTIONS INTO PAG OR MRF (mm^3)

Case	Tracer	Injection Center	Center + Diffusion Zone
L-STT/MRF			
124	RhS	0.350	2.642
130	RhS	0.208	0.359
131	RhS	0.156	0.769
132	RhS	0.231	0.777
133	RhS	0.171	0.511
136*	RhS	0.200	0.871
L-STT/PAG			
501	RhS	0.219	2.084
502	RhS	0.238	2.403
503	RhS	0.147	1.729
504 *	RhS	0.080	0.390
505	RhS	0.094	0.780
M-STT/MRF			
126	RhS	0.666	2.893
128	RhS	0.215	2.497
129 *	RhS	0.343	1.484
139	RhS	0.153	0.605
140	RhS	0.146	0.407
142	RhS	0.139	1.046
M-STT/PAG			
301	RhS	0.394	2.387
303 *	RhS	0.226	0.844
312	RhS	0.070	0.315
314	RhS	0.080	0.287
315	RhS	0.092	0.220

*Case is individually illustrated

TABLE 6

SUMMARY OF DATA FROM AREAS USED AS CHECKPOINTS FOR PAG OR MRF INJECTIONS

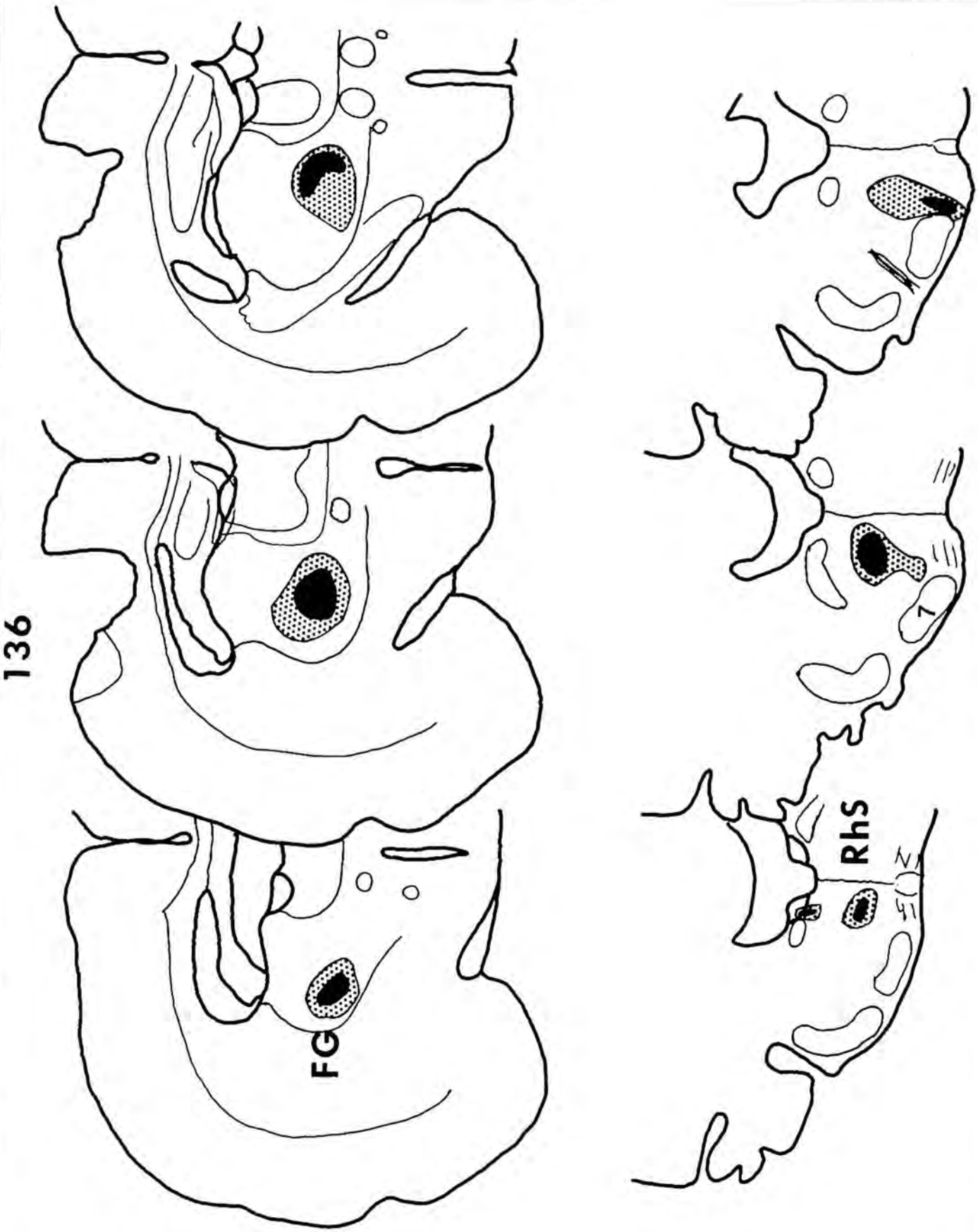
Case	N. Gracilis	N. Cuneatus
L-STT/MRF		
124	-	-
130	-	-
131	+	+
132	-	-
133	-	-
136 *	+	+
L-STT/PAG		
501	-	-
502	-	+
503	-	-
504 *	-	+
505	-	-
M-STT/MRF		
126	++	++
128	-	-
129 *	-	-
139	-	-
140	+	+
142	-	-
M-STT/PAG		
301	-	+
303 *	-	-
312	-	-
314	-	-
315	-	-

\+ 3-10 cells labeled
++ 11-20 cells labeled
+++ 21-30 cells labeled
++++ more than 31 cells labeled in one 40 µm section. Data is pooled from 3-5 sections.

* Case is individually illustrated

FIGURE 6

Line drawings of the FG and RhS injection sites from a L-STT/MRF case (136). The centers of each injection are shown in black and the diffusion zones are represented by the shaded regions. For each site, the middle drawing is a tracing of the section with the largest injection center. The most rostral and caudal sections in which the central zone of each injection was present are depicted in the right and left drawings respectively.


136
FG
RhS

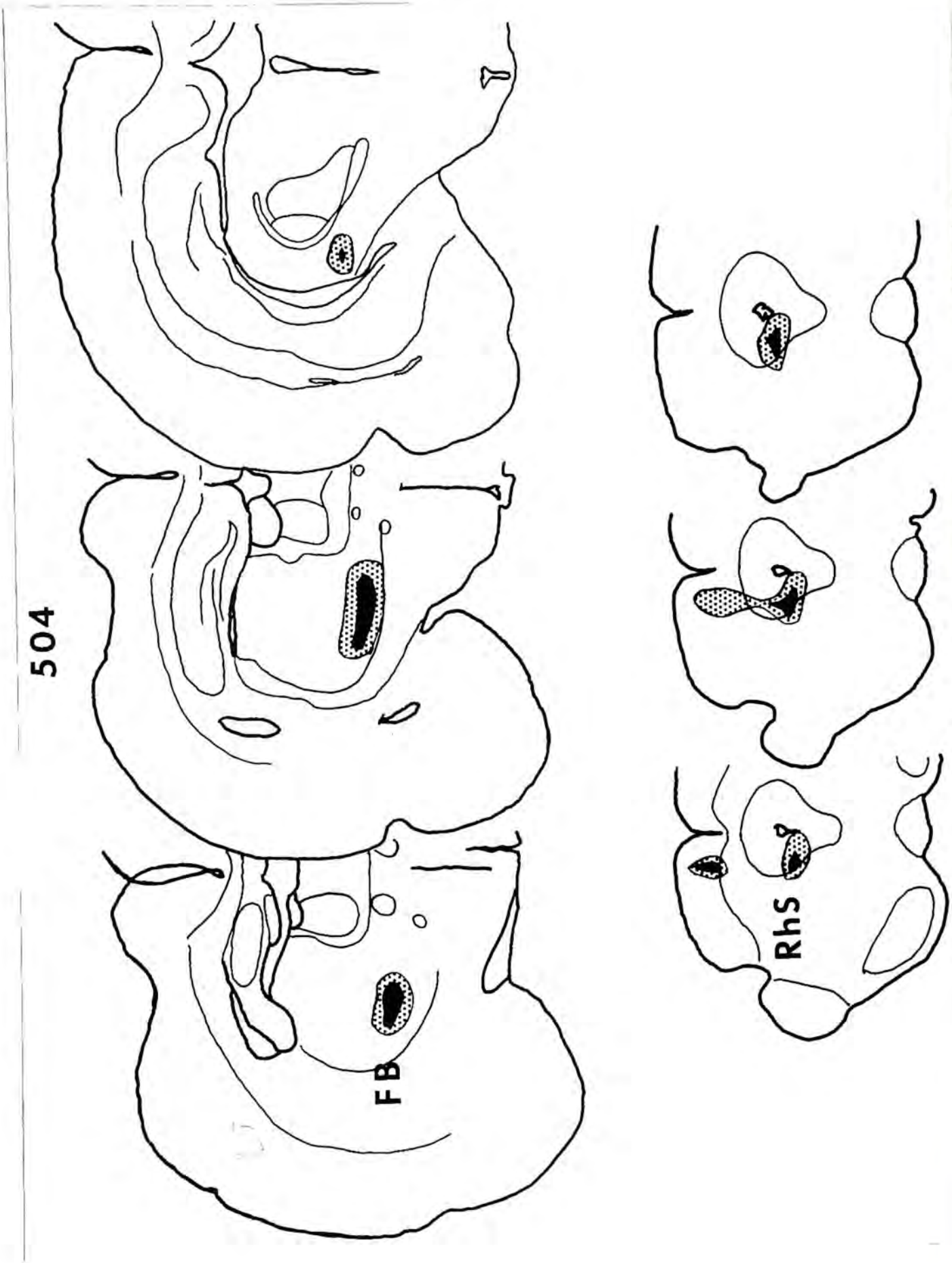
posterior and ventromedial nuclei. A photomicrograph of the middle part of this thalamic injection is shown in Figure 10 (A). The MRF injection was centered in the n. reticularis gigantocellularis with some spread into n. reticularis magnocellularis ventrally and caudally. A small area, diffusion in the pipette track, was seen rostrally in the n. reticularis paragigantocellularis dorsalis. A photomicrograph of this injection is shown in Figure 10 (B).

The injection sites of a L-STT/PAG case (504) are depicted in Figure 7. The thalamic injection of FB was centered more caudally than in the previous case. The ventral parts of the VPL and ventromedial nuclei were the most involved by this injection as can be seen in the photomicrograph in Figure 10 (C). The rostral limit of the injection core extended into the ventral lateral nucleus. The caudal limit extended as a small spot into the ventral part of the lateral geniculate nucleus. This spot of tracer seemed to surround the basal lamina of a blood vessel. There was only a small amount of the posterior nucleus involved in the diffusion zone of this injection. The lateral PAG in case 504 contained tracer from the rostral to the mid-superior collicular levels. A small area of diffusion into the pipette track was observed in the rostral superior colliculus; however, only the diffusion zone was apparent at the mid-point of the injection. A photomicrograph of this injection is shown in Figure 10(D).

The injection sites of a M-STT/MRF case (129) are

FIGURE 7

Line drawings of the FB and RhS injection sites from a L-STT/PAG case (504). The centers of each injection are shown in black and the diffusion zones are represented by the shaded regions. For each site, the middle drawing is a tracing of the section with the largest injection center. The most rostral and caudal sections in which the central zone of the injections was present are depicted in the right and left drawings respectively.


504
FB
RhS

shown in Figure 8. The center of the thalamic injection involved the central lateral nucleus throughout most of its rostrocaudal extent. At its largest point the injection impinged upon the central lateral, mediodorsal, and paracentral nuclei with the diffusion zone spreading into the medial part of the posterior nucleus. A photomicrograph of this site is included in Figure 11 (A). The MRF injection in this animal was similar, but somewhat rostral, to that in case 136. It was centered in the n. reticularis gigantocellularis but did not involve the n. reticularis magnocellularis. This injection can be seen in the photomicrograph in Figure 11 (B).

Finally, Figure 9 depicts the injection sites in a M-STT/PAG case (303). A large thalamic injection of FB exhibited spread of the central zone into the medial part of the posterior nucleus. However, the central core of the injection did involve the central lateral, paracentral and mediodorsal nuclei. Figure 11 (C) shows a photomicrograph of this injection. The PAG injection in 303 was quite small and was located almost entirely within the lateral PAG. It extended from the very rostral portion of the superior colliculus to the mid-superior collicular level. This injection site is shown in Figure 11 (D).

FIGURE 8

Line drawings of the FB and RhS injection sites from a M-STT/MRF case (129). The centers of each injection are shown in black and the diffusion zones are represented by the shaded region. For each site, the middle drawing is a tracing of the section with the largest injection center. The most rostral and caudal sections in which the central zone of the injections was present are depicted in the right and left drawings respectively.

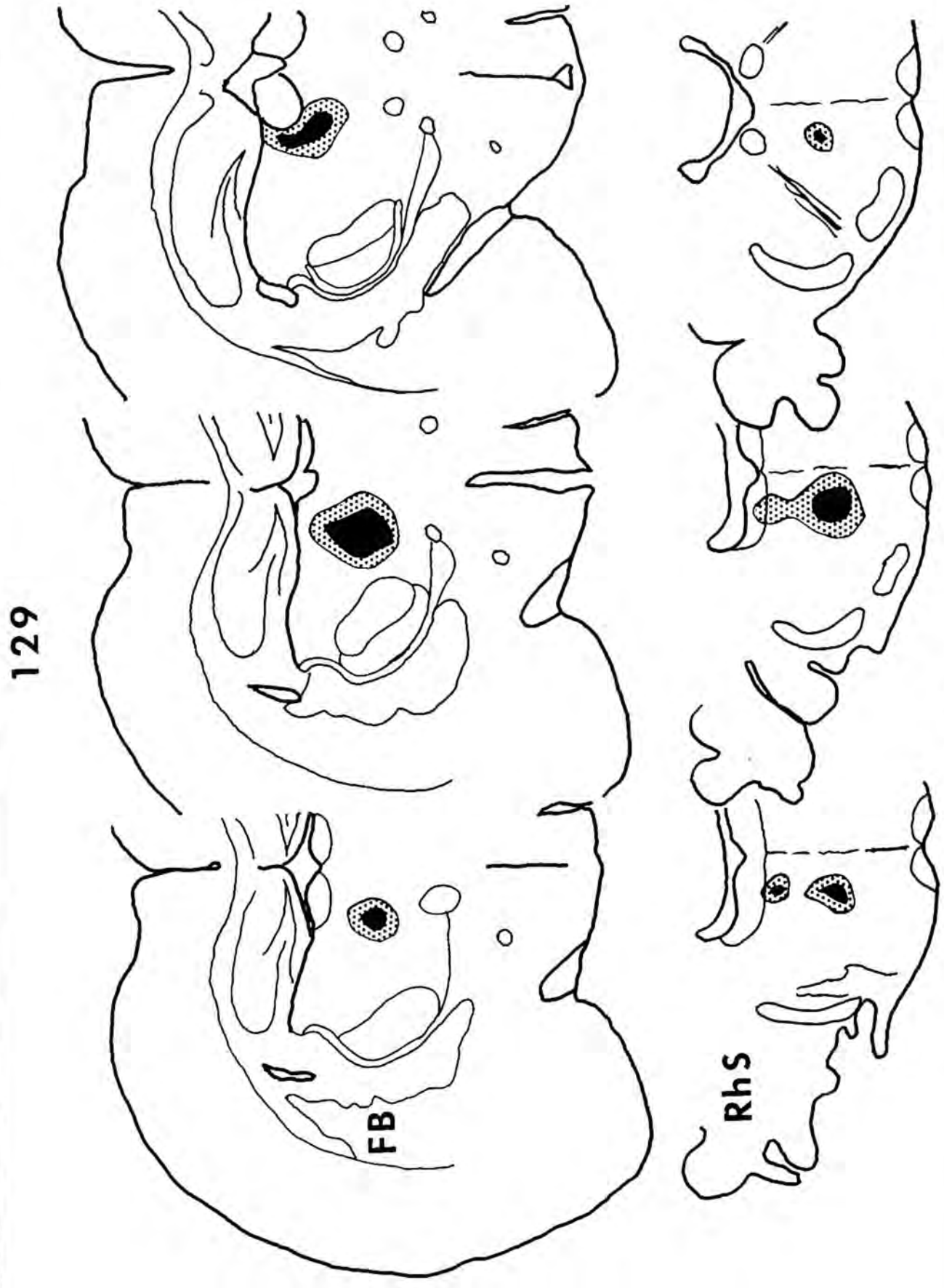

129
FB
RhS

FIGURE 9

Line drawings of the FB and RhS injection sites from a M-STT/PAG case (303). The centers of each injection are shown in black and the diffusion zones are represented by the shaded region. For each site, the middle drawing is a tracing of the section with the largest injection center. The most rostral and caudal sections in which the central zone of the injections was present are depicted in the right and left drawings respectively.

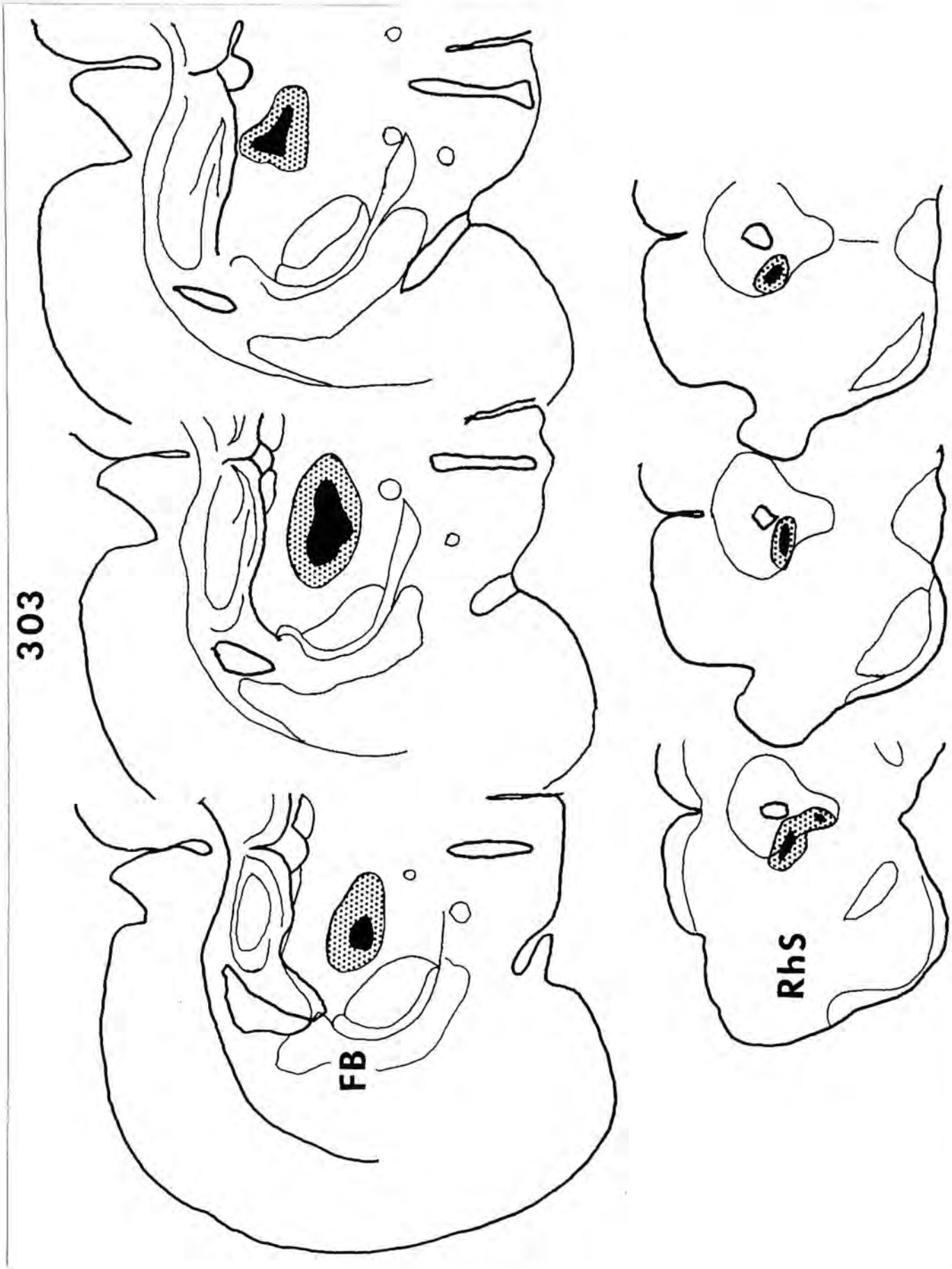

303
FB
RhS

FIGURE 10

Photomicrographs of the injection sites in cases 136 (A,B,) and 504 (C,D). These sections are either the same or alternate sections from those used for the middle drawings in Figures 6 and 7. In A (19X) and C (19X), the lateral thalamic injections of FG and FB respectively were photographed under 420 nm wavelength light. The MRF (B, 29X) and PAG (D, 19X) injections of RhS were photographed under 550 nm wavelength light.

FIGURE 10

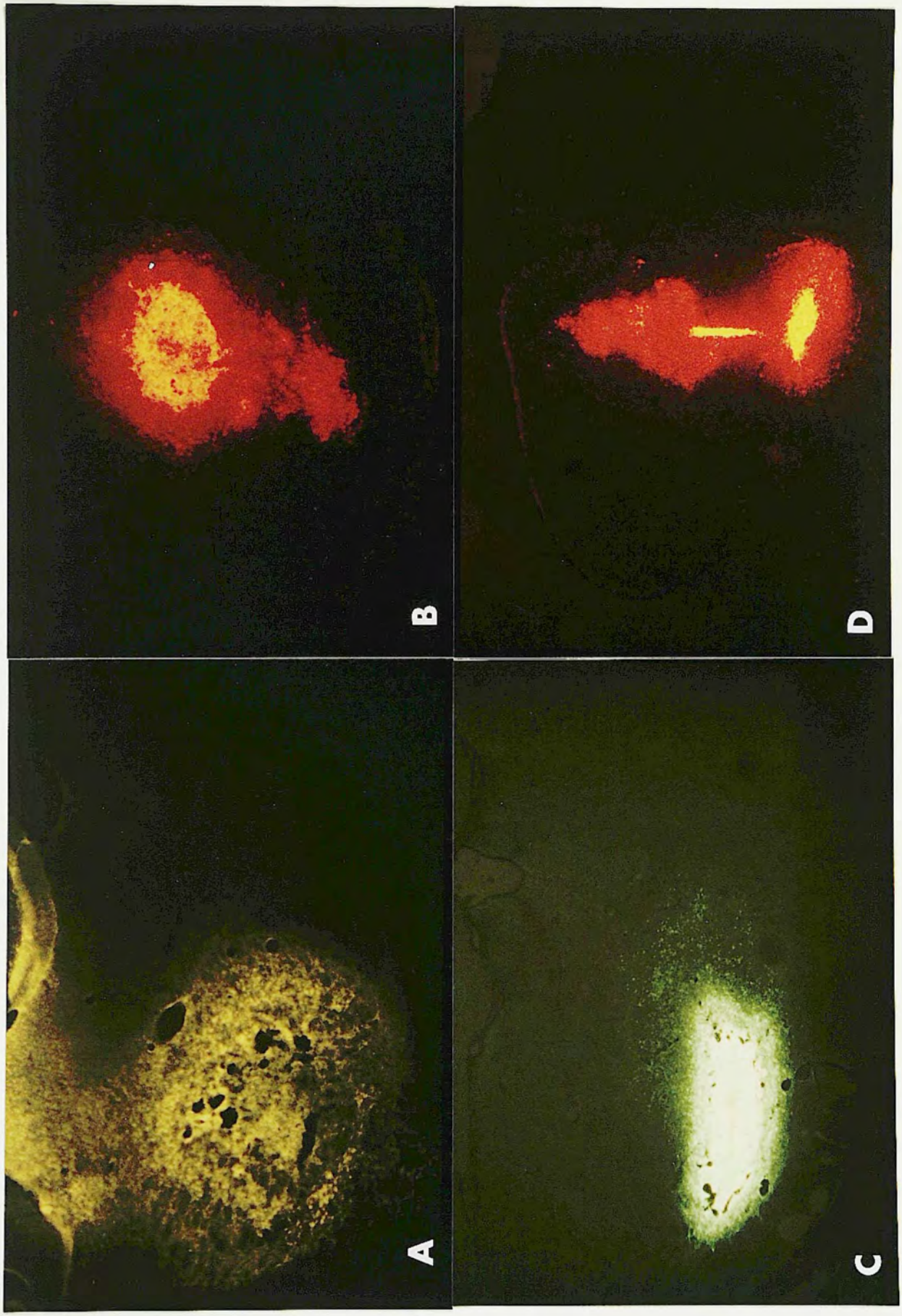
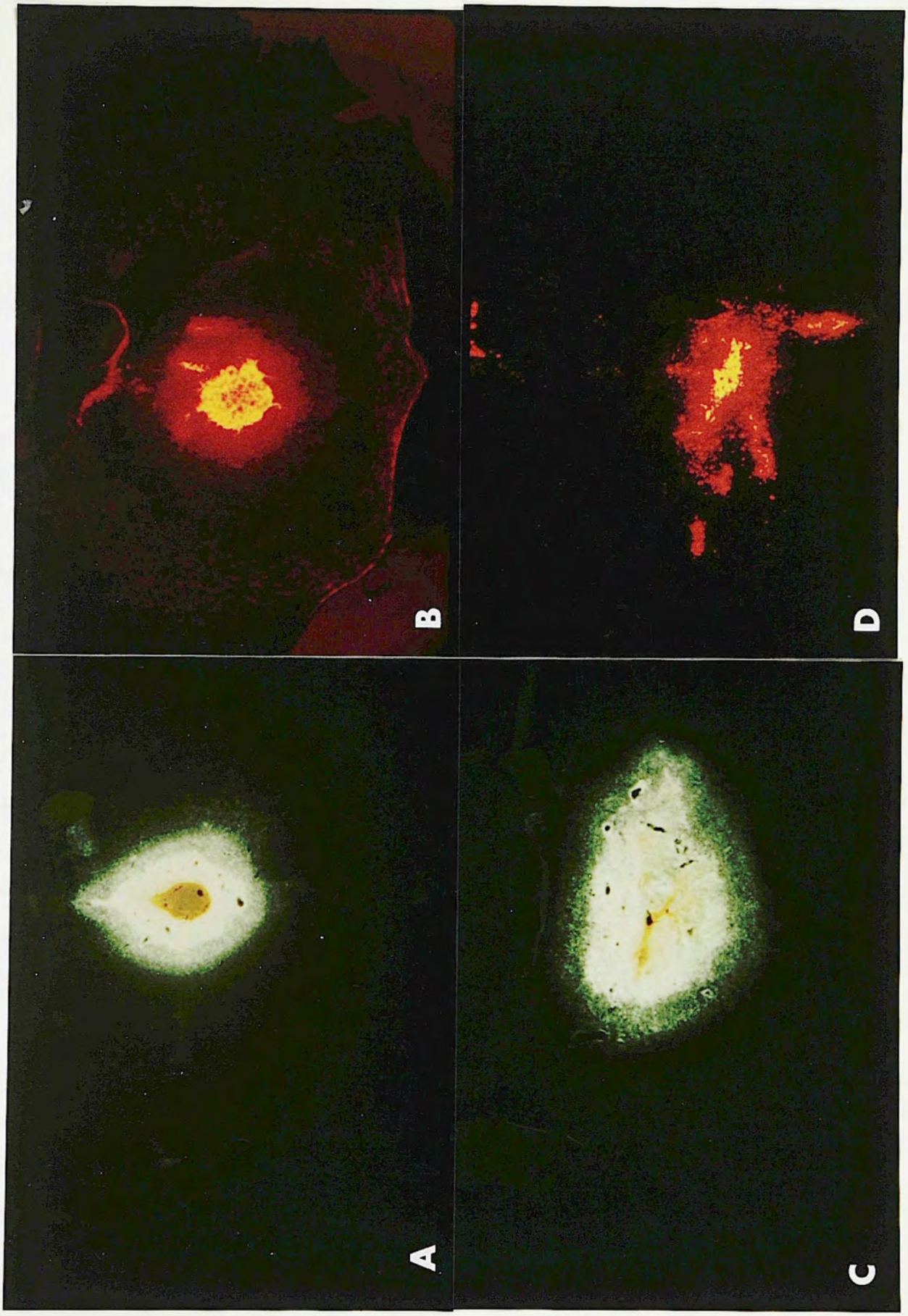


FIGURE 11

Photomicrographs of the injection sites in cases 129 (A,B) and 303 (C,D). These sections are either the same or alternate sections from those used for the middle drawings in Figures 8 and 9. In A and C (19X), the medial thalamic injections of FB were photographed under 420 nm wavelength light. The MRF (B, 19X) and PAG (D, 29X) injections of RhS were photographed under 550 nm wavelength light.

FIGURE 11



DOUBLE-LABEL STUDIES: SPINAL LABELING

Characteristics of labeled neurons

Spinal neurons were single-labeled with FB, FG or RhS or were double-labeled with either FB and RhS or FG and RhS. All three of these tracers were found in the cytoplasm and were usually excluded from the nuclei. FB-labeled neurons exhibited blue or green fluorescent cytoplasm when viewed under 365nm or 420nm wavelength light respectively. The labeling was homogeneous and non-granular. Figure 12 (A,B) presents examples of neurons single-labeled with FB. Under these same wavelengths of light, the cytoplasm of FG-labeled cells was either pale gold (365nm) or yellow (420nm). FG labeling was often granular in appearance, especially in lightly to moderately filled cells. Compared to FB, FG often was visible in smaller dendrites and, thus, revealed greater morphological detail of labeled cells. Examples of FG-labeled neurons are shown in Figure 12 (C,D). RhS-labeled neurons were seen under 550nm wavelength light. The cytoplasm of such cells contained brilliant red granules as can be seen in Figure 12 (E). Such cells, lightly or moderately labeled with RhS, were almost always visible only with high magnification (25-40X).

The individual characteristics of these tracers were not changed in double-labeled neurons, examples of which are shown in Figure 13. Every cell labeled from the thalamus (FB or FG) was examined at 40X magnification for the

FIGURE 12

Photomicrographs of spinal neurons (742X) single-labeled with FB (A,B), FG (C,D) and RhS (E). FB-labeled neurons were blue under 365 nm wavelength light (A) and green under 420 nm wavelength light (B, same neuron as in A). FG-labeled neurons were pale gold (C) or yellow (D) under these respective wavelengths of light. Note the more granular nature of FG labeling compared to FB. RhS-labeled neurons exhibited bright red granules under 550 nm wavelength light (E).

FIGURE 12

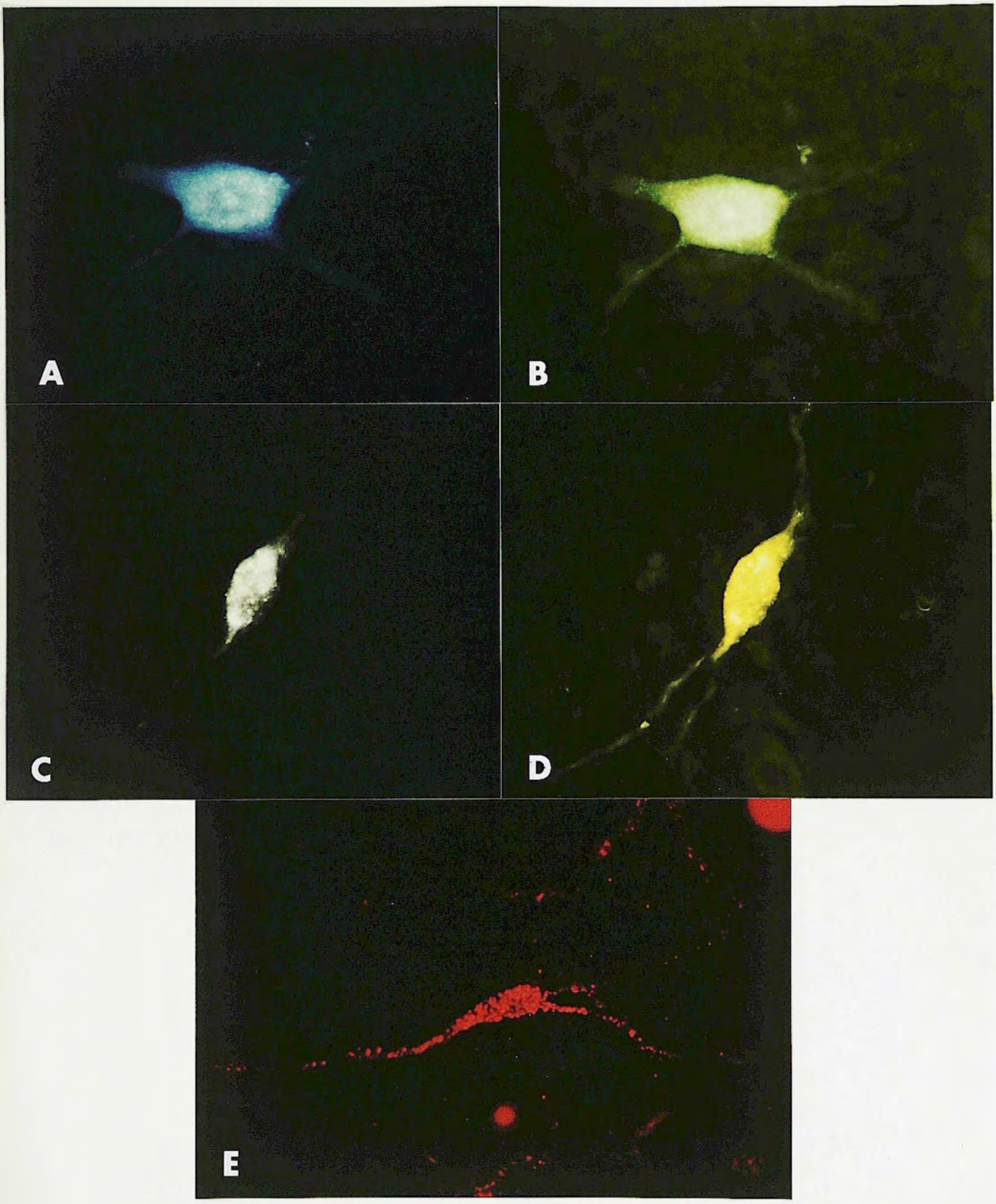
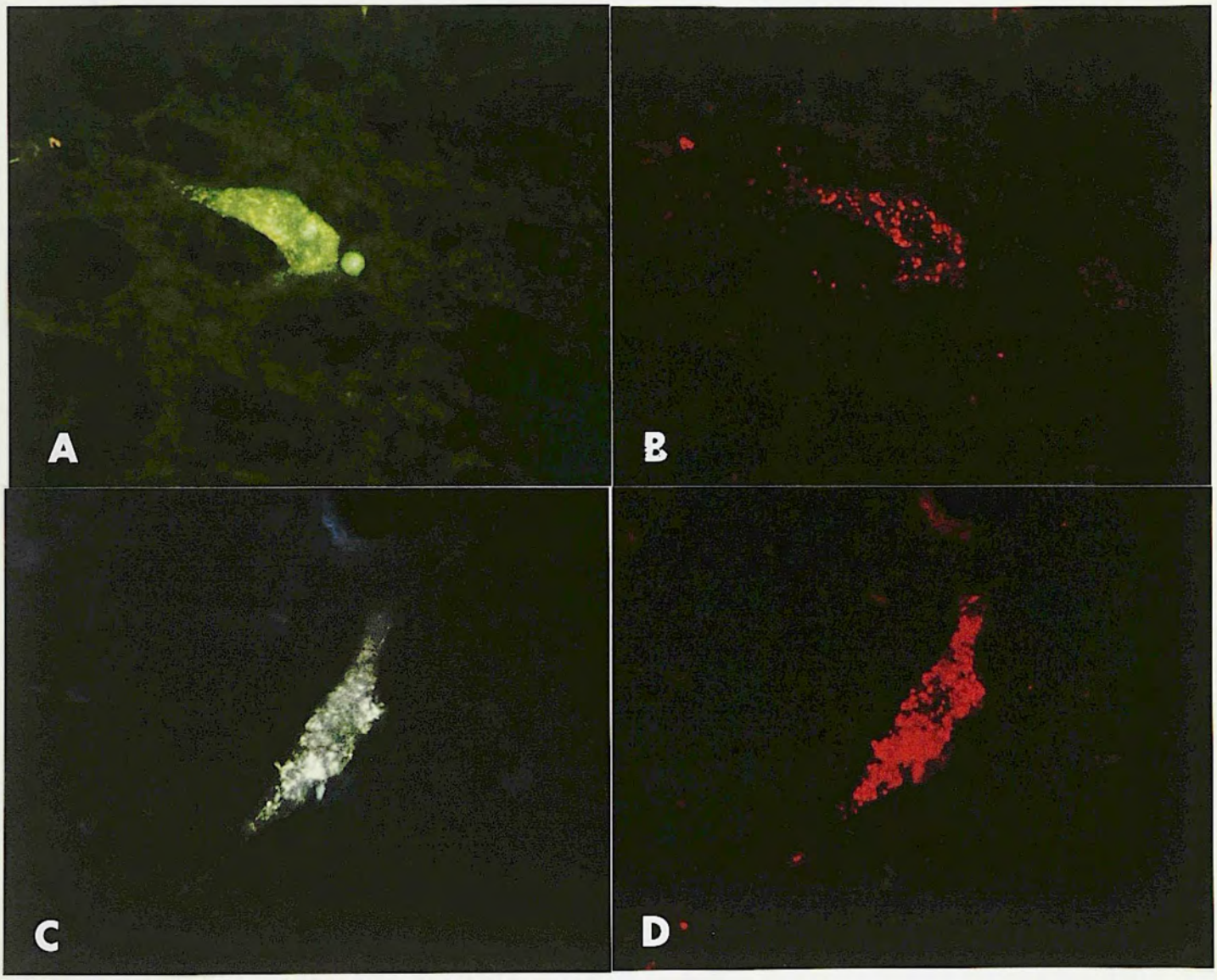


FIGURE 13

Photomicrographs of spinal neurons (742X) which were double-labeled with FB (A) and RhS (B) or FG (C) and RhS (D). The granular nature of FG labeling can be difficult to distinguish from RhS labeling in black and white photographs especially if the RhS labeling is heavy. This problem does not occur with FB since the difference in the texture of labeling between FB and RhS is quite clear even in black and white photographs. The neuron in A and B was located in contralateral lamina V of the upper cervical segments in case 1005, a triple-label case. In C and D, the neuron was observed in case 136 (L-STT/MRF group) in the lumbar enlargement in the contralateral lamina VII.

FIGURE 13



presence of RhS double-labeling. Since both tracers in a double-labeled cell could not be seen simultaneously, a cell had to contain sufficient quantities of each tracer to identify the morphological features of the neuron. Normally, thirty or more granules of RhS were required to make such an identification.

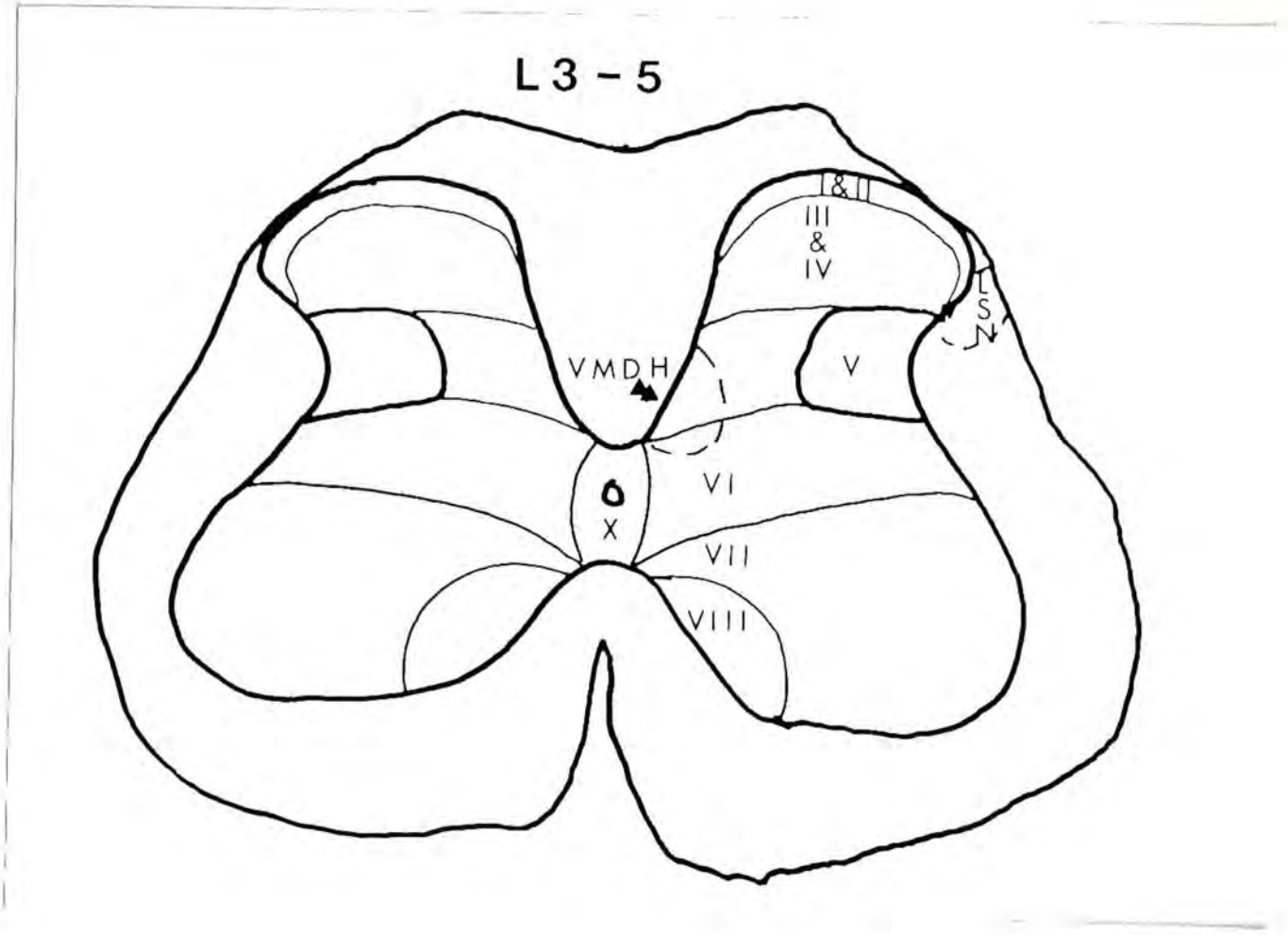
Lumbar Enlargement

The laminar divisions of the lumbar enlargement used in these studies is presented in Figure 14. These divisions represent a combination of the laminae described by Rexed (1952,1954) with those defined by Paxinos and Watson (1982). Two additional regions are included in Figure 14 which are not represented in either Paxinos and Watson's or Rexed's definitions; these are the lateral spinal nucleus (LSN) and the ventromedial dorsal horn (VMDH). The LSN (Baker and Giesler,1984;Giesler and Elde,1985) corresponds to the nucleus of the dorsolateral funiculus of Gwyn and Waldron (1968,1969). The VMDH is a loosely defined region which has been discussed in previous STT studies (Giesler et al.,1981;Menetrey et al.,1984b,1985). The use of fluorescent tracers precludes the use of counter-stains such as cresyl violet. Therefore discrimination of laminar boundaries is less precise. Some laminar boundaries, between laminae VII and VIII for example, have in fact been reported to be very gradual and not clearly apparent in the rat even in counter-stained tissue (McClung and

FIGURE 14

Line drawing of a transverse spinal section illustrating the laminar boundaries used in the present study for the lumbar enlargement.

FIGURE 14



Castro,1978). The locations of labeled cells were mapped using the following landmarks: the central canal, the reticulated portion of the dorsal horn, the lateral and medial extent of the gray matter, blood vessels and other features of each section.

Differential labeling of STT neurons following medial versus lateral thalamic injections can be seen in Table 7. L-STT and M-STT neurons were observed, contralateral to the injection sites, in all laminae of the lumbar enlargement except for laminae II, III and IX. More M-STT neurons were observed ipsilateral to the injection sites, and in the LSN bilaterally, than L-STT neurons. There were only a few labeled cells, L-STT or M-STT, in laminae I, IV, and X.

In the lateral, reticulated part of lamina V and in laminae VI, VII and VIII, both L-STT and M-STT labeled neurons were common. However, in the VMDH, many neurons were labeled from the lateral thalamic injections whereas only a few were labeled following medial injections. L-STT injections labeled more cells in the dorsal horn (laminae I-V, LSN and VMDH) than in the ventral gray (laminae VI-VII and X). The average ratio of cells labeled in the dorsal gray matter to those labeled in the ventral gray matter for the L-STT groups was 2.1 $\pm$ 0.1 ($\bar{X}\pm$S.E.M.). This ratio was 0.9 $\pm$ 0.1 for the M-STT groups.

The pattern of labeling of spinoreticular (SRT) and spinoannular (SAT) tract neurons is summarized in Table 8.

TABLE 7

NUMBER OF STT NEURONS LABELED: LUMBAR ENLARGEMENT

$\bar{X} \pm$ S.E.M., Number labeled in 50 (25μm) sections from each case

LAMINA	L-STT (N=11)	M-STT (N=11)
CONTRALATERAL		
I	2.3 ± 0.8	4.1 ± 1.6
V	19.6 ± 2.5	11.0 ± 1.2
VI, VII, VIII	29.6 ± 2.4	26.4 ± 2.2
X	4.9 ± 1.4	4.8 ± 0.7
LSN	3.4 ± 0.9	11.7 ± 2.1
VMDH	46.9 ± 4.5	1.6 ± 0.3
IPSILATERAL		
I	0.4 ± 0.3	1.6 ± 0.8
V	2.5 ± 0.7	4.9 ± 1.0
VI, VII, VIII	1.2 ± 0.4	5.6 ± 0.9
X	0.6 ± 0.3	2.5 ± 0.8
LSN	2.0 ± 1.1	10.4 ± 2.6
VMDH	-	-

TABLE 8

NUMBER OF NEURONS LABELED FROM THE PAG OR MRF: LUMBAR ENLARGEMENT

$\bar{X} \pm$ S.E.M., Number labeled in 30 (25μm) sections from each case

LAMINA	SRT (N=12)	SAT (N=10)
CONTRALATERAL		
I	2.7 ± 1.2	10.3 ± 3.0
V	23.7 ± 3.5	11.9 ± 2.0
VI, VII, VIII	42.8 ± 9.0	13.4 ± 3.6
X	11.8 ± 2.5	5.6 ± 0.8
LSN	10.8 ± 2.9	10.9 ± 2.6
VMDH	-	-
IPSILATERAL		
I	-	-
V	26.8 ± 4.9	5.8 ± 1.9
VI, VII, VIII	15.7 ± 2.7	2.0 ± 1.0
X	5.2 ± 1.4	2.6 ± 0.9
LSN	11.9 ± 2.5	5.5 ± 1.5
VMDH	-	-

In general, more neurons were labeled following MRF injections than PAG injections. Spinal neurons projecting to the MRF were observed bilaterally in laminae V (lateral portion), and bilaterally with contralateral predominance in laminae VI, VII, VIII, and X. There were a few such neurons in lamina I on the contralateral side only. These same laminae contained neurons labeled from the PAG injections. However, more neurons were labeled in lamina I from the PAG than from the MRF and neurons in lamina V labeled from the PAG were located mostly on the contralateral side. There were very few and sometimes no neurons labeled in the VMDH from MRF or PAG injections. Finally, neurons labeled from the PAG or MRF were present in the lateral spinal nucleus. Those labeled from the PAG exhibited a contralateral predominance, whereas those labeled from the MRF were present bilaterally.

Comparison of the standard errors of the means of Table 7 and Table 8 indicates greater variability between the cases in terms of RhS labeling versus FB labeling. This greater variability exists despite the observation that RhS injections were more uniform than FB injections based on tissue volume and injection site mapping data (see Tables 3 and 5 for comparison).

Before considering the double-label data, it is important to clarify two points. First, since the purpose of this study was to distinguish differences between L-STT and M-STT neurons in terms of their collateral targets, it

is most important to look at the proportion of STT neurons which were double-labeled rather than looking at the percentage as a function of all the labeled cells from both injection sites. The second point relates to the qualitative nature of these data. There are many sources of unavoidable variability in these kinds of studies including differences in injection volume, injection placement, transport of tracers and normal biological variability between animals. In double-label studies, two injections of two different tracers are made; thus, the potential for variation from some of these sources is at least doubled. Therefore, the cell counts and, especially, the percentages of double-labeled cells should be viewed as qualitative measures. The data are presented, in part, by tables and histograms which are not intended as a quantitative analysis but, rather, as a means of organization and clarification.

The percentage of STT neurons which were double-labeled is presented in Table 9 for the contralateral side and in Table 10 for the ipsilateral side. As is apparent from these tables, double-labeled neurons in most laminae were not observed in every animal. This was especially true for regions of sparse labeling such as lamina I, X and the LSN. In contrast, in the contralateral laminae V-VIII most animals in all four groups exhibited double-labeled neurons. There were slightly higher percentages of double-labeled STT neurons in the MRF groups than in the PAG groups; however, this could simply be due to better labeling

TABLE 9 PERCENT OF STT NEURONS DOUBLE-LABELED: CONTRALATERAL LUMBAR ENLARGEMENT

%
(DL/SL+DL STT CELLS, n=NUMER OF CASES WITH DL CELLS)

LAMINAE	L-STT/MRF (N=6)	L-STT/PAG (N=5)	M-STT/MRF (N=6)	M-STT/PAG (N=5)
I	-	29% (5/17, n=3)	-	8% (2/24, n=2)
V	23% (21/90, n=5)	11% (13/117, n=5)	25% (13/51, n=5)	17% (7/42, n=3)
VI, VII, VIII	10% (14/137, n=5)	15% (18/124, n=4)	27% (34/125, n=6)	9% (12/135, n=4)
X	56% (9/15, n=3)	-	32% (6/19, n=4)	29% (4/14, n=3)
LSN	-	28% (5/18, n=3)	19% (6/31, n=4)	15% (13/88, n=5)
VMDH	-	-	-	-

TABLE 10 PERCENT OF STT NEURONS DOUBLE-LABELED: IPSILATERAL LUMBAR ENLARGEMENT

% (DL/SL&DL STT CELLS, n = NUMBER OF CASES WITH DL CELLS)

LAMINAE	L-STT/MRF (N=6)	L-STT/PAG (N=5)	M-STT/MRF (N=6)	M-STT/PAG (N=5)
I	-	-	-	-
V	-	-	22% (2/9, n=2)	21% (5/24, n=3)
VII, VIII	33% (2/6, n=2)	-	32% (10/31, n=5)	-
X	-	-	-	-
LSN	-	29% (2/7, n=2)	24% (5/21, n=2)	6% (2/35, n=2)
VMDH	-	-	-	-

following MRF injections. Both L-STT and M-STT neurons in lamina I were double-labeled from the PAG, but not from the MRF. Some double-labeled L-STT and M-STT neurons were observed ipsilateral to the injection sites. Finally,there was a striking absence of double-labeled L-STT neurons in the VMDH.

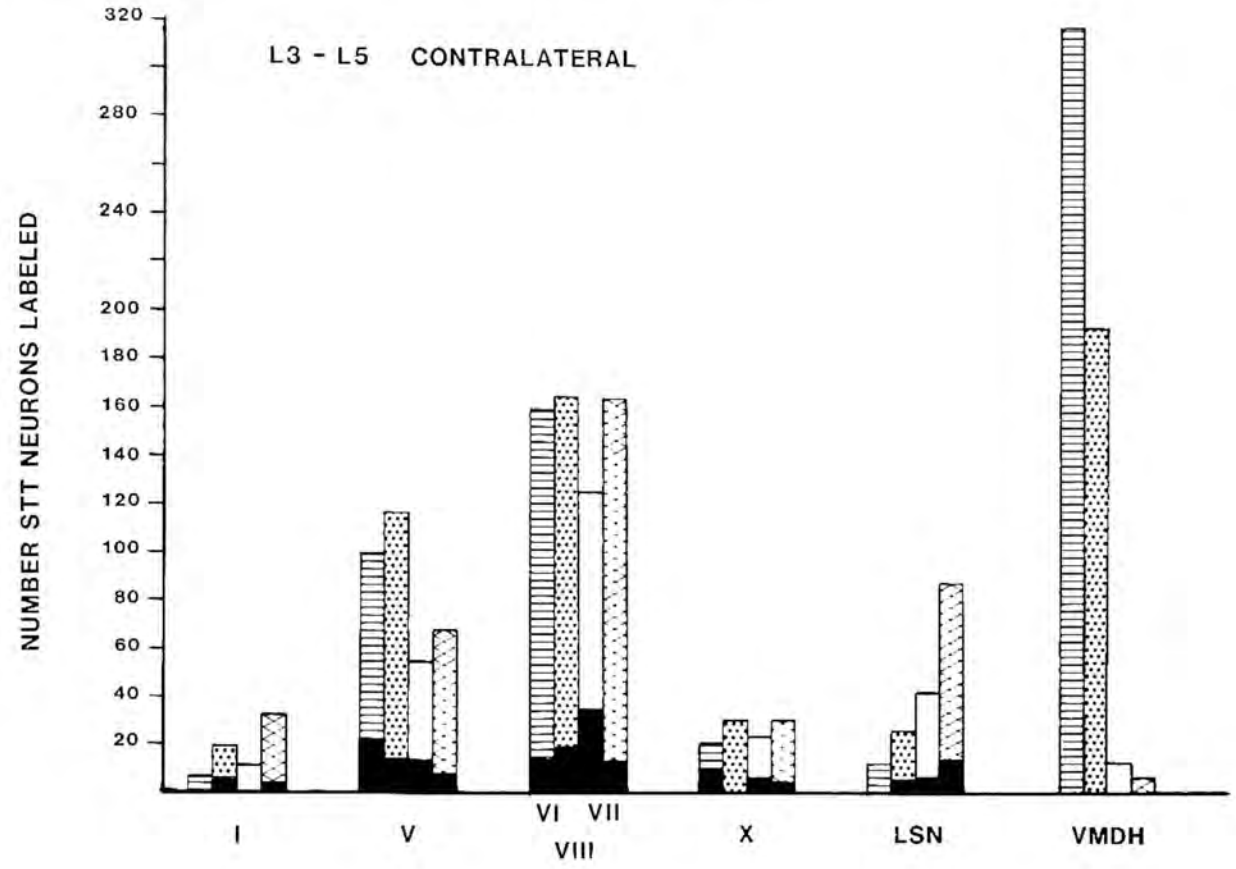
The total number of single- and double-labeled STT neurons is presented in histogram form for each of the four groups in Figure 15. In this form, the percentages of double-labeled STT neurons are somewhat lower since all the cases of each group are included regardless of whether they contained any double-labeled neurons in specific laminae. Nevertheless, it is clear that double-labeled STT neurons were present in all four groups. These neurons tend to be located in laminae V-VIII and in the LSN. STT neurons in lamina I were double-labeled only from the PAG. The only region which contained neurons which seem to project directly, without collaterals, to the thalamus is the VMDH and these cells were labeled from the lateral thalamus. VMDH neurons may project to the ventral lateral nucleus of the thalamus since cases with extensive labeling in the VMDH were those in which there was involvement of the ventral lateral nucleus in the thalamic injection site.

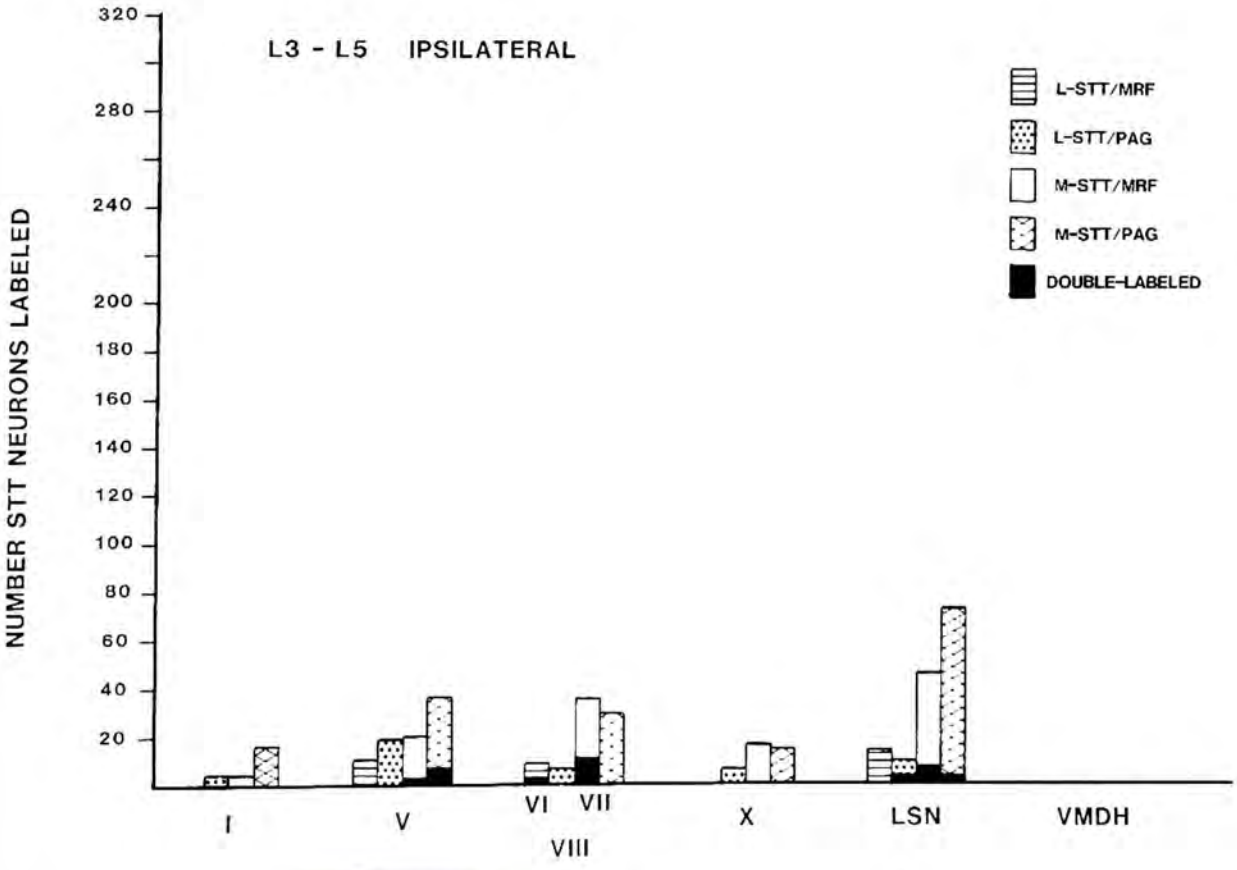
The spinal labeling data from the lumbar enlargement of an individual case from each of the four groups are illustrated in Figures 16-19. The injection sites for these cases were presented above (Figures 6-11). Case 136 (Fig

FIGURE 15

Histograms showing the total number of STT neurons in specific spinal laminae which were single- and double-labeled in each experimental group in the lumbar enlargement. The data for each group are pooled from: 6 animals in the L-STT/MRF group; 5 in the L-STT/PAG group; 6 in the M-STT/MRF group; and 5 in the M-STT/PAG group.

FIGURE 15


L3 - L5 CONTRALATERAL
NUMBER STT NEURONS LABELED
320
280
240
200
180
160
140
120
100
80
60
40
20
I
V
VI VII
VIII
X
LSN
VMDH


L3 - L5 IPSILATERAL
NUMBER STT NEURONS LABELED
L-STT/MRF
L-STT/PAG
M-STT/MRF
M-STT/PAG
DOUBLE-LABELED
320
280
240
200
180
160
140
120
100
80
60
40
20
I
V
VI VII
VIII
X
LSN
VMDH

16) received tracer injections into the lateral thalamus and MRF. Double-labeled L-STT neurons were located in laminae V, VI, VII, and VIII contralateral to the injection sites. There was one double-labeled cell in the ipsilateral lamina V. In this particular case, no cells which contained both FG and RhS were observed in the LSN although there were both single-labeled L-STT and spinoreticular neurons present in this region. Typical of the L-STT groups, there was a heavy concentration of labeled L-STT neurons contralaterally in the VMDH, none of which were double-labeled.

A L-STT/PAG case (504) is illustrated in Figure 17. Double-labeled neurons were present in laminae V-VII but also in lamina I and the LSN on the contralateral side. In addition, L-STT neurons containing a RhS double-label were observed in lamina X and in the ipsilateral LSN and lamina VIII. Many L-STT neurons, single-labeled, were again seen in the contralateral VMDH.

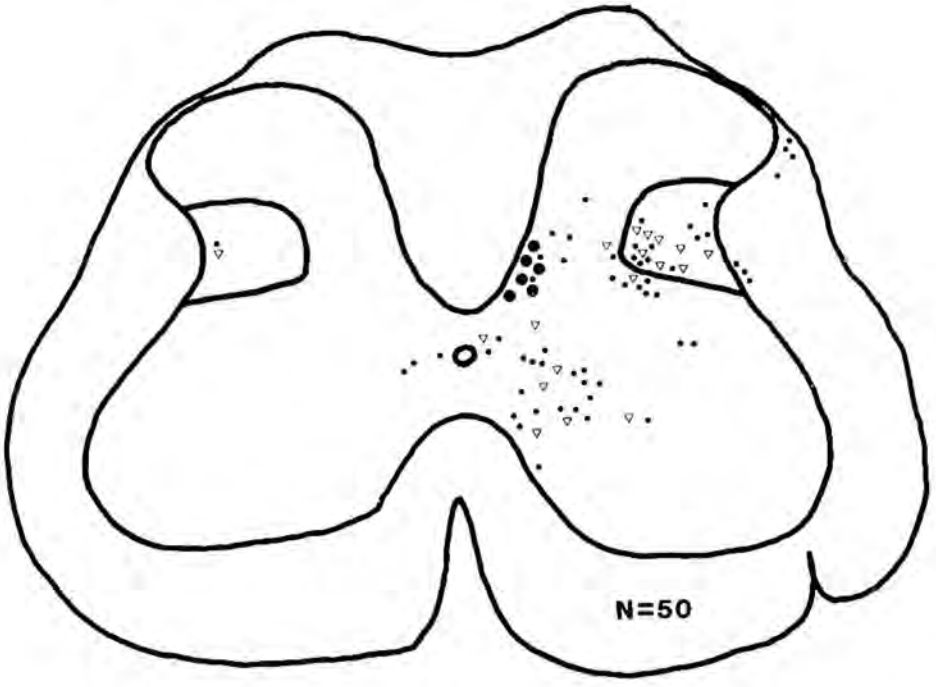
Comparison of the single- and double-labeled L-STT neurons shown in Figures 16 and 17 reveals some of the variability observed between cases. For example, there is greater bilateral labeling in case 504. Also, case 504 presented single-labeled cells in lamina IV. In general, few STT cells were seen in lamina IV. In the absence of counter-staining, the most prominent landmark in the dorsal horn is the reticulated part of lamina V through which fiber bundles traverse the gray matter in a rostro-caudal manner. It was not uncommon to observe labeled STT neurons

FIGURE 16

Line drawings illustrating the number and locations of single- and double-labeled neurons in the lumbar enlargement of case 136, a L-STT/MRF case. Labeled L-STT neurons are shown in the top drawing and neurons labeled from the MRF are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The large dots represent 10 single-labeled neurons. The right side of each drawing is contralateral to the injection sites.

136

L-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SRT CELLS

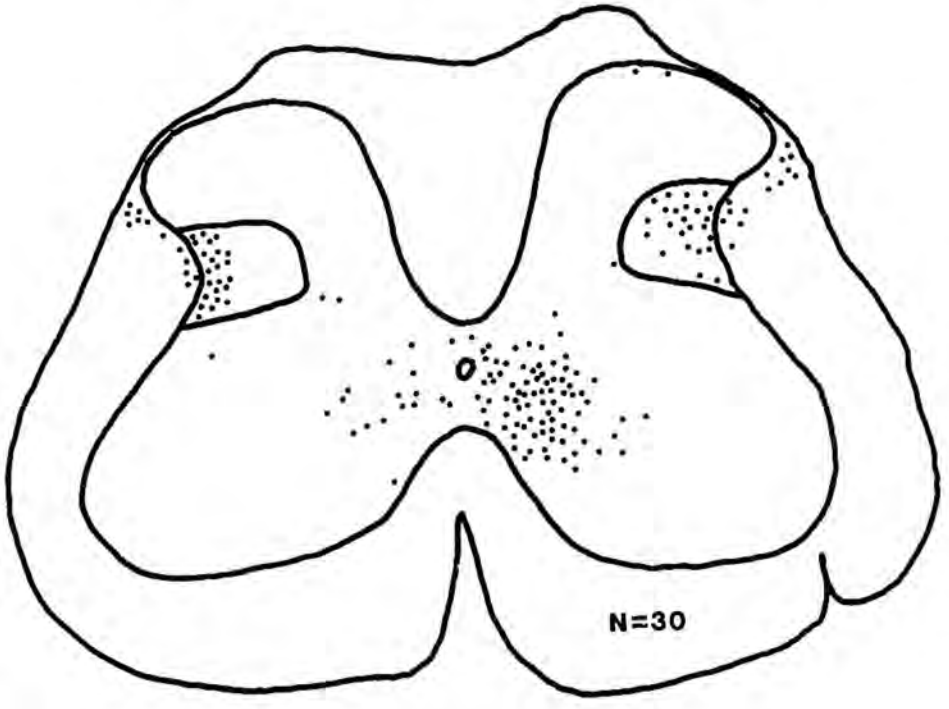
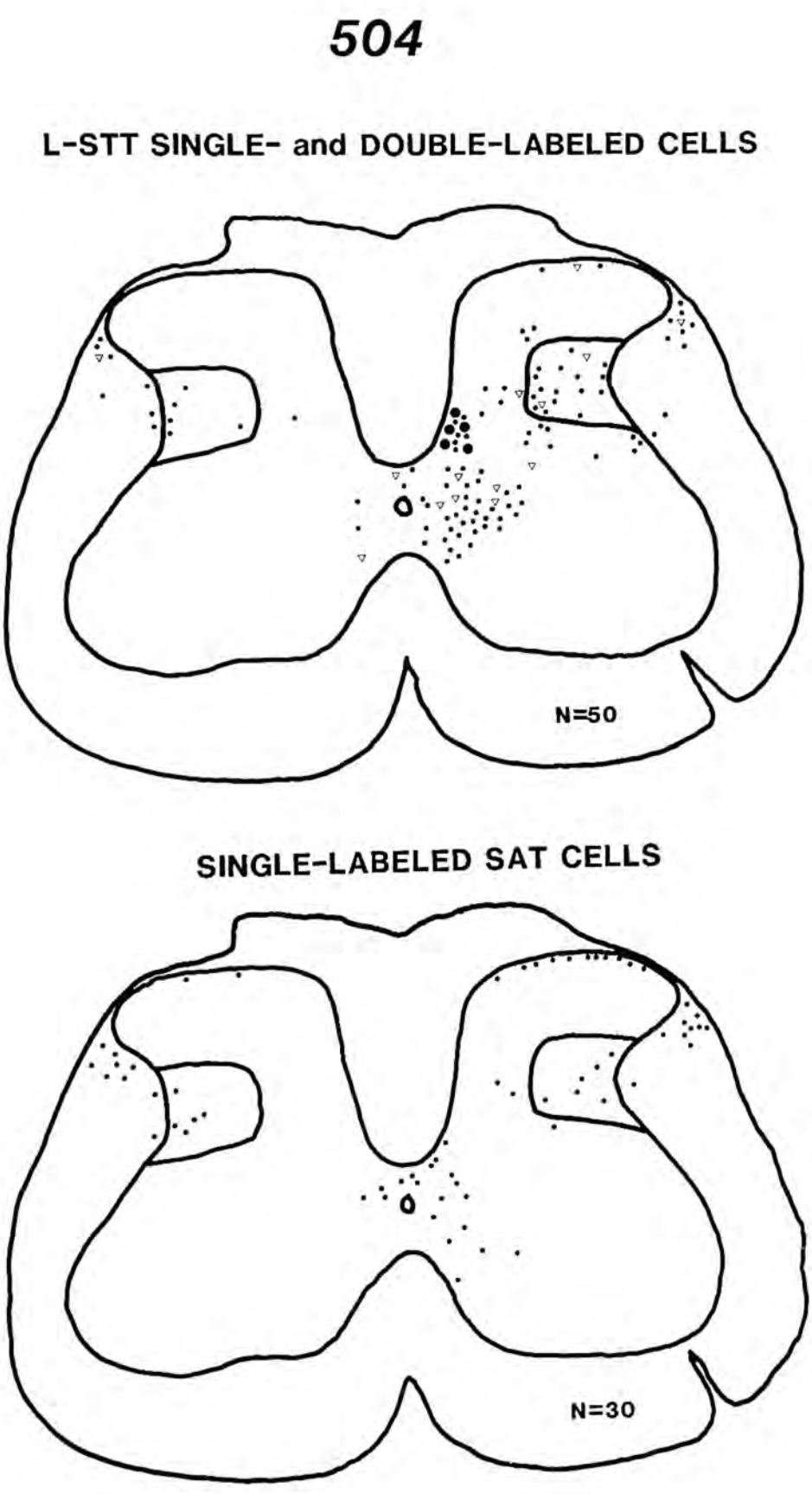


FIGURE 17

Line drawings illustrating the number and locations of single- and double-labeled neurons in the lumbar enlargement of case 504, a L-STT/PAG case. Labeled L-STT neurons are shown in the top drawing and neurons labeled from the PAG are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The large dots represent 10 single-labeled neurons. The right side of each drawing is contralateral to the injection sites.


504
L-STT SINGLE- and DOUBLE-LABELED CELLS
N=50
SINGLE-LABELED SAT CELLS
N=30

in the most dorsal part of this reticulated substance. Such neurons were assigned to lamina V. Labeled cells which were clearly outside this region and dorsal to it were assigned to lamina IV. It is possible that some true, lamina IV, STT neurons were included in lamina V due to the lack of clear boundary definition in tissue prepared for fluorescent tracing. Finally, the thalamic injection site in 504 was considerably more ventral and caudal than that in 136. Case 504's injection site was unusual among cases with heavy VMDH labeling in that it only minimally involved the ventral lateral nucleus. However, it was possibly ventral and caudal enough to involve axons in passage to the ventral lateral nucleus.

Figure 18 shows the data obtained from a M-STT/MRF case (129). In this animal, double-labeled M-STT neurons were observed in the LSN, laminae V, VI and VII on the contralateral side. Ipsilaterally, only one such cell was seen in lamina VIII. In the contralateral VMDH, a few single-labeled M-STT neurons were seen.

A M-STT/PAG case (303) is illustrated in Figure 19. Double-labeled M-STT neurons were observed bilaterally in the LSN and in lamina V. Contralaterally, double-labeled neurons were located in laminae VII and VIII.

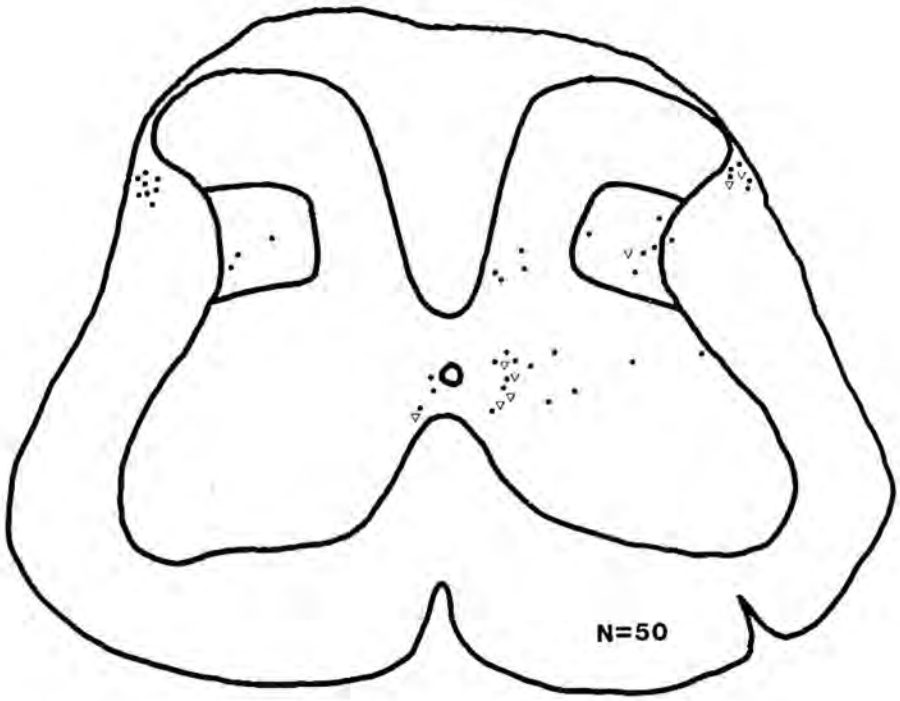
In summary, in the lumbar enlargement, L-STT and M-STT neurons which issue axon collaterals to the PAG or MRF are located in the contralateral laminae V-VIII and the LSN. The few STT neurons labeled in lamina I were double-

FIGURE 18

Line drawings illustrating the number and locations of single- and double-labeled neurons in the lumbar enlargement of case 129, a M-STT/MRF case. Labeled M-STT neurons are shown in the top drawing and neurons labeled from the MRF are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

129

M-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SRT CELLS

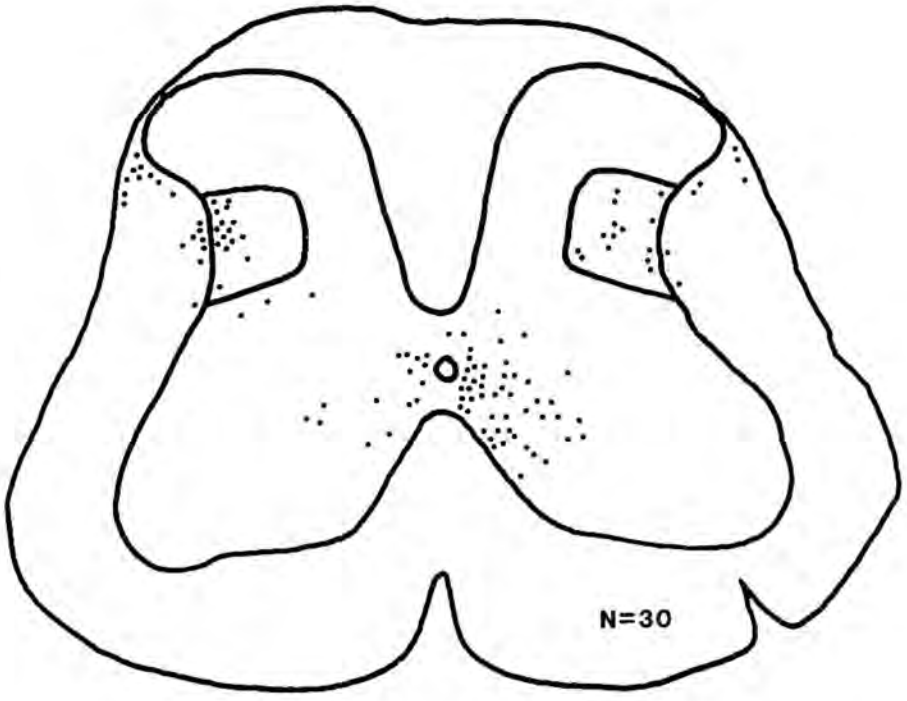
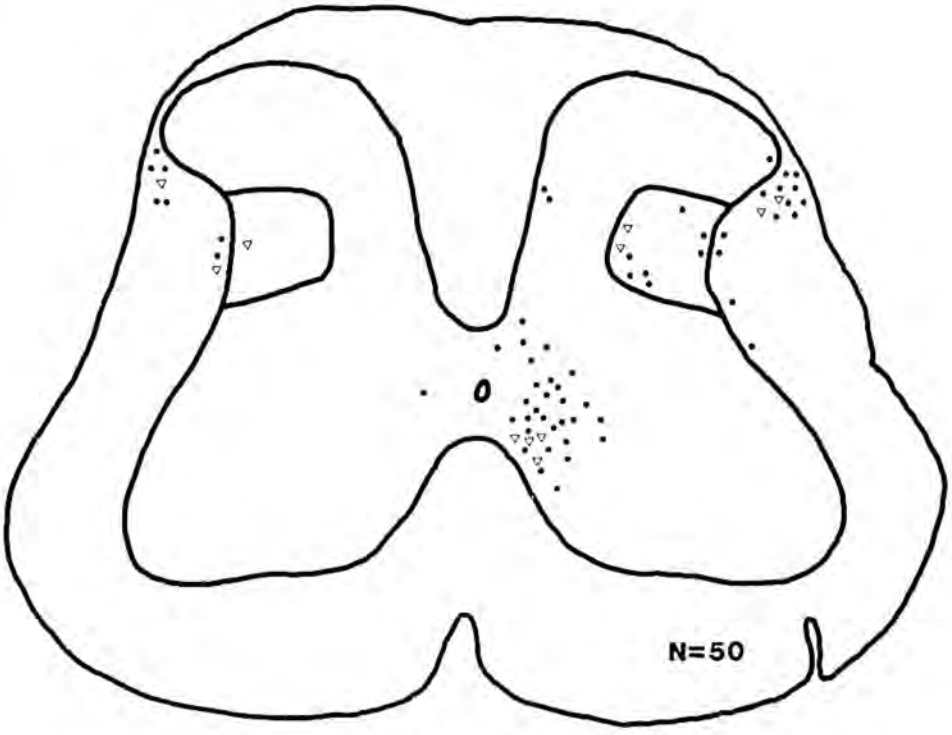


FIGURE 19

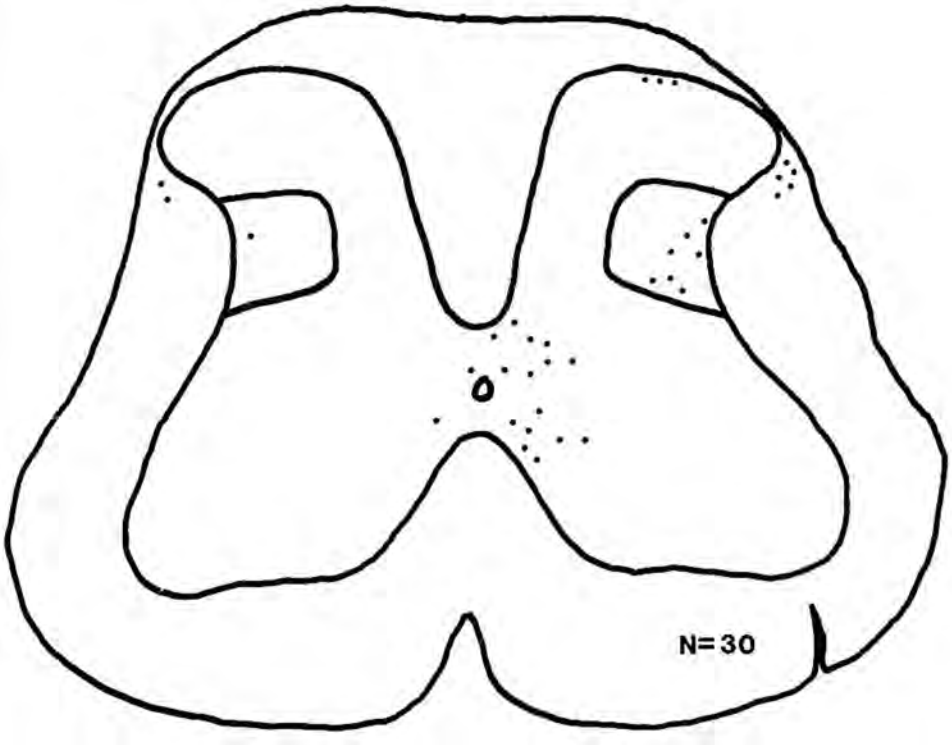
Line drawings illustrating the number and locations of single and double-labeled neurons in the lumbar enlargement of case 303, a M-STT/PAG case. Labeled M-STT neurons are shown in the top drawing and neurons labeled from the PAG are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

303

M-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SAT CELLS



labeled only from the PAG. Somewhat more double-labeled M-STT neurons were observed ipsilateral to the injection sites than L-STT neurons. RhS injections into the MRF resulted in more labeled cells than RhS injections into the PAG. The contralateral VMDH was the only region in which exclusively single-labeled STT neurons were found and the vast majority of these were L-STT cells. Photomicrographs of double-labeled L-STT and M-STT neurons are presented in Figure 20. Examples of L-STT neurons in the VMDH are shown in Figure 21.

Cervical Enlargement

The laminar delineations used for the cervical enlargement are illustrated in Figure 22. These boundaries are from Paxinos and Watson (1982) with the addition of the LSN. Unlike the lumbar enlargement, there is no region in the cervical enlargement which corresponds to the VMDH.

Differences in labeling between L-STT and M-STT groups are summarized in Table 11. Both L-STT and M-STT neurons were located bilaterally in laminae I, V-VIII, X, and in the LSN but, as in the lumbar segments, there was a strong contralateral predominance. There were more L-STT neurons labeled in the contralateral lamina I than M-STT cells. In contrast, M-STT cells were slightly more numerous in the LSN and laminae VI,VII, and VIII. Also, there were few neurons, L-STT or M-STT, labeled in lamina X. Finally, M-STT groups exhibited more ipsilateral labeling than L-STT

FIGURE 20

Photomicrographs (742X) of three double-labeled neurons in the lumbar enlargement. In A and B, a neuron containing FB (A, 420 nm light) and RhS (B, 550 nm light) is shown. This neuron was located in lamina VII, contralateral to the injection sites, in case 124 (L-STT/MRF). A neuron found in case 504 (L-STT/PAG) is shown in C (FB) and D (RhS). This cell was also found in contralateral lamina VII. There is only one double-labeled neuron in E and F (arrows) which contained FB (E) and RhS (F). The neuron at the top was so heavily labeled with RhS that some of this label showed through under 420 nm wavelength light (E). Both cells were located in the lateral spinal nucleus of case 124 (L-STT/MRF), ipsilateral to the injection sites.

FIGURE 20

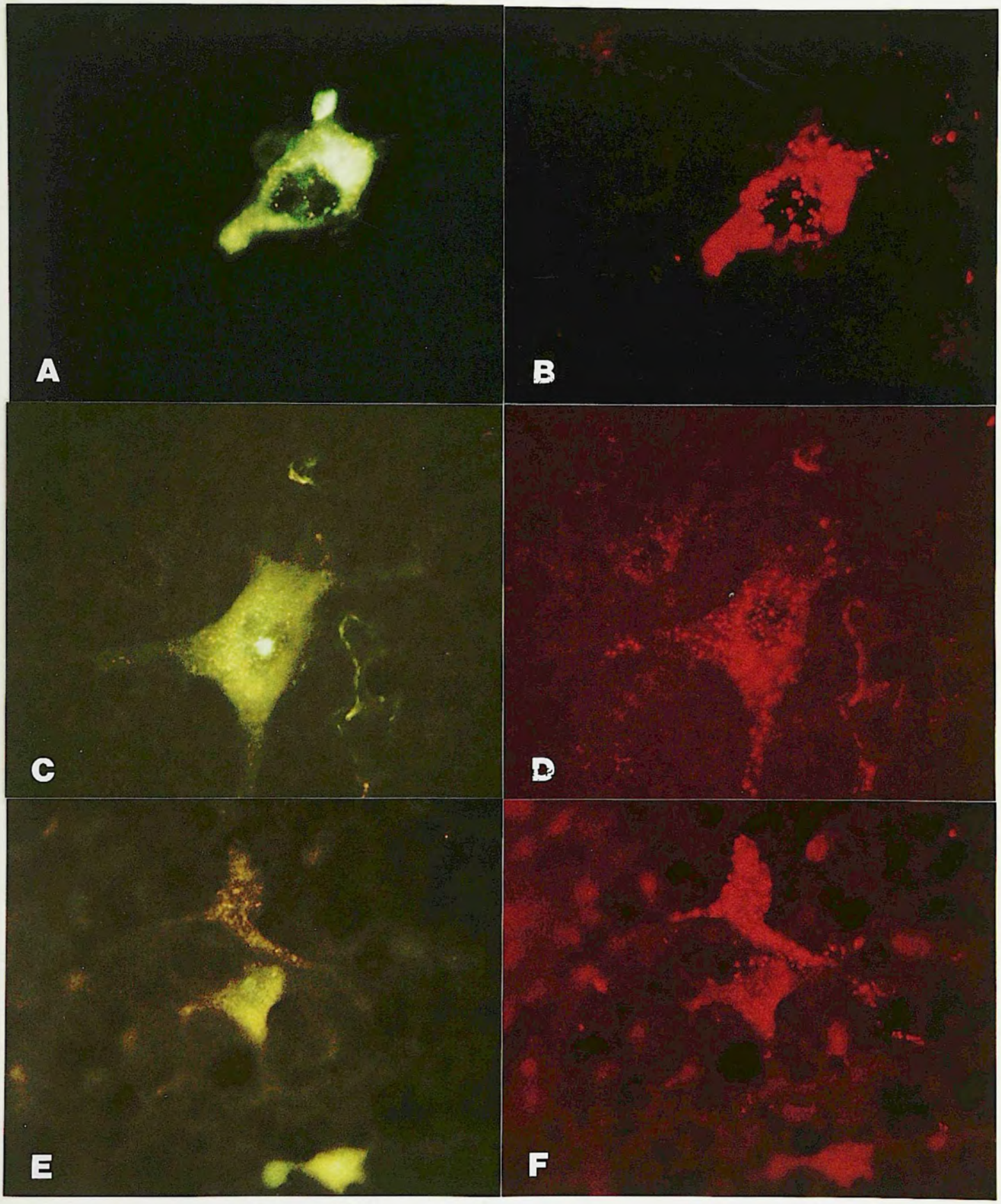


FIGURE 21

Photomicrographs of cells in the ventromedial dorsal horn of case 136 (L-STT/MRF). In A, a low power view (93X) of this region is presented showing three FG-labeled neurons in the same section (420 nm wavelength light). In B and C, higher magnifications (234X and 594X respectively) were used to illustrate cellular detail. Both photomicrographs in B and C were taken under 365 nm wavelength light.

FIGURE 21

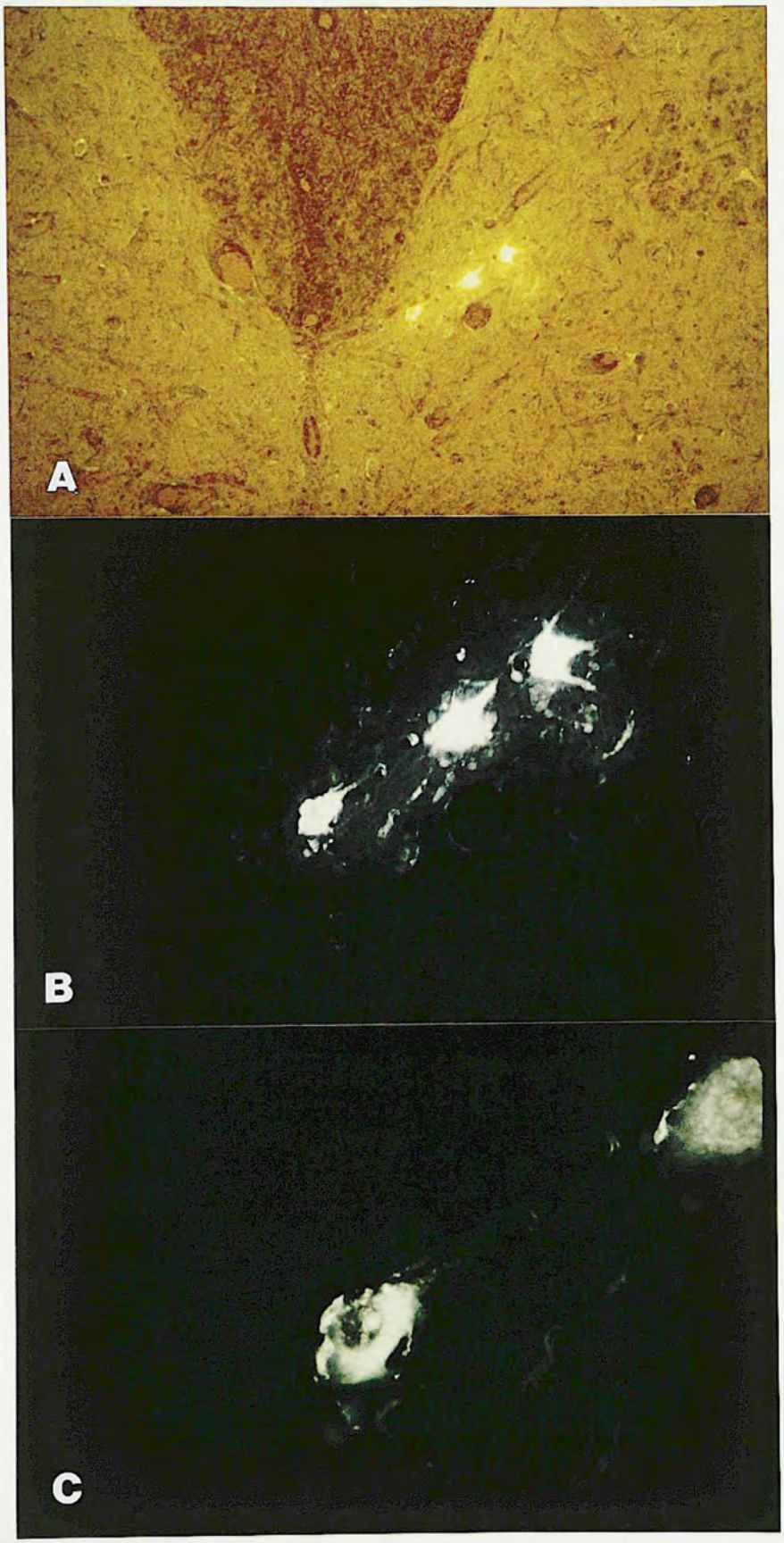


FIGURE 22

Line drawing of a transverse spinal section illustrating the laminar boundaries used in the present study for the cervical enlargement.

FIGURE 22

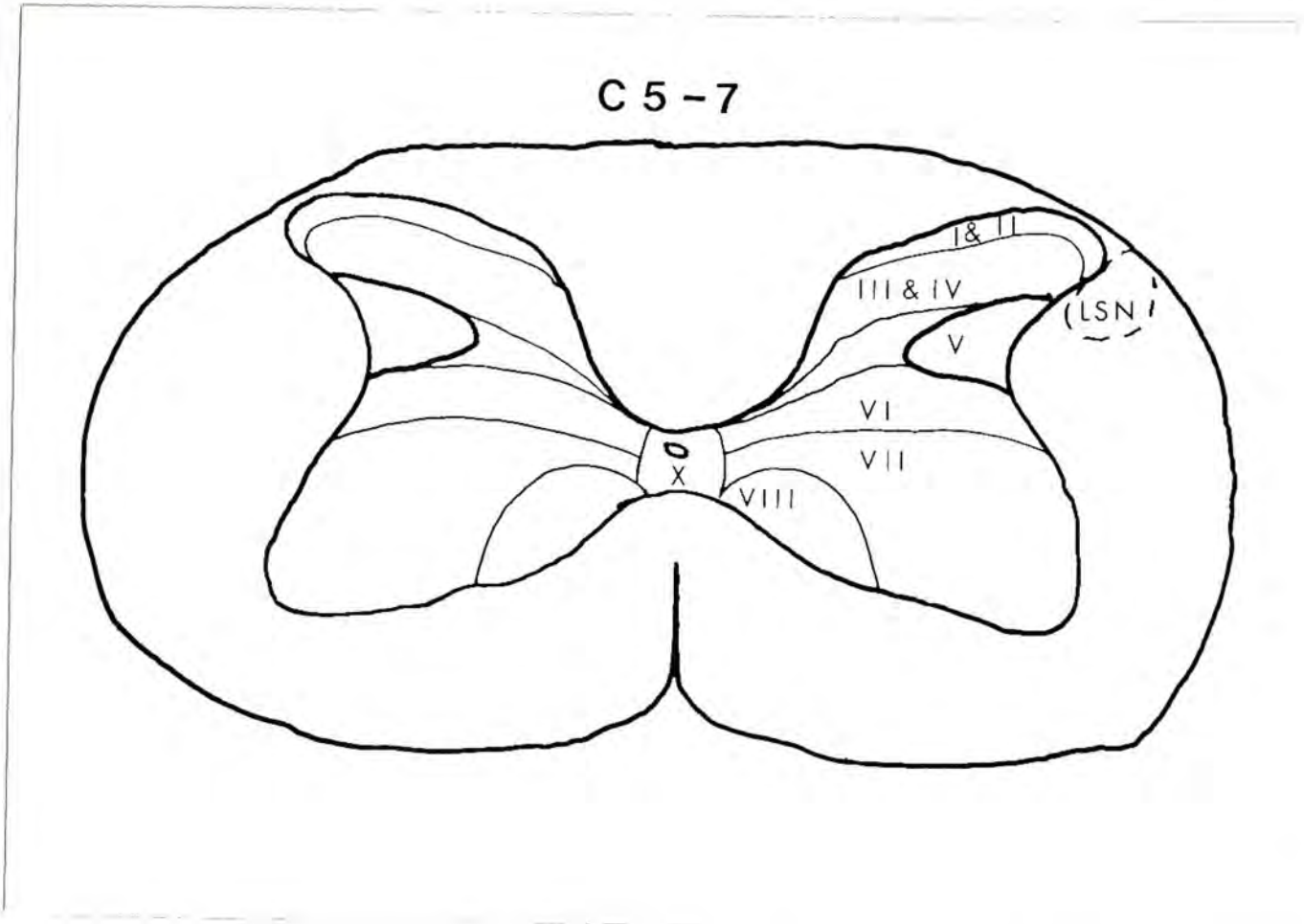


TABLE 11

NUMBER OF STT NEURONS LABELED: CERVICAL ENLARGEMENT

$\bar{X} \pm$ S.E.M., Number labeled in 50 (25μm) sections from each case

LAMINAE	L-STT (N=11)	M-STT (N=11)
CONTRALATERAL		
I	24.0 ± 6.8	8.5 ± 2.0
V	11.3 ± 2.8	14.1 ± 2.6
VI, VII, VIII	9.1 ± 1.8	16.6 ± 2.0
X	1.3 ± 0.4	1.6 ± 0.6
LSN	9.1 ± 2.9	15.4 ± 3.0
IPSILATERAL		
I	1.4 ± 0.5	2.0 ± 0.9
V	4.0 ± 1.3	6.9 ± 0.8
VI, VII, VIII	1.5 ± 0.5	5.7 ± 1.0
X	0.4 ± 0.3	1.5 ± 0.5
LSN	2.8 ± 1.1	10.0 ± 1.9

groups, similar again to the lumbar enlargement.

Besides the absence of the VMDH, certain differences in labeling were apparent in the comparison of lumbar and cervical enlargements. For example, there were more cells labeled in cervical lamina I, especially L-STT neurons, but there were fewer STT neurons of either type labeled in cervical laminae VI-VIII.

The pattern of labeling following PAG and MRF injections is presented in Table 12. There were many more spinoreticular neurons labeled than spinoannular neurons. However, both types were present in laminae I,V,VI-VIII,X, and the LSN. Lamina I contained neurons most of which were labeled from the PAG in a strongly contralateral distribution. Spinoreticular neurons were present almost equally in lamina V of both sides and with contralateral predominance in laminae VI-VIII and X; whereas spinoannular cells exhibited contralateral predominance in lamina V as well as in laminae VI-VIII and X. In the LSN, there was a bilateral distribution of spinoreticular neurons but twice as many spinoannular neurons were found on the contralateral versus the ipsilateral side. Except for the observation of greater numbers of labeled cells in the cervical enlargement, the pattern of labeling of spinoreticular and spinoannular neurons was essentially the same as that seen in the lumbar enlargement.

Tables 13 and 14 present the data regarding the percentage of STT neurons which were double-labeled on the

TABLE 12

NUMBER OF NEURONS LABELED FROM THE PAG OR MRF: CERVICAL ENLARGEMENT

$\bar{X} \pm$ S.E.M., Number labeled in 30 (25 µm) sections from each case

LAMINAE	SRT (N=12)	SAT (N=10)
CONTRALATERAL		
I	3.3 ± 1.5	28.9 ± 6.7
V	45.3 ± 5.4	11.6 ± 3.0
VI, VII, VIII	76.0 ± 10.1	6.6 ± 1.8
X	19.2 ± 5.2	2.0 ± 0.7
LSN	9.8 ± 2.5	16.5 ± 3.6
IPSILATERAL		
I	0.8 ± 0.3	3.7 ± 1.1
V	39.1 ± 7.8	5.5 ± 1.5
VI, VII, VIII	39.4 ± 5.5	1.8 ± 0.6
X	5.1 ± 1.7	0.8 ± 0.3
LSN	9.3 ± 1.9	8.1 ± 1.7

TABLE 13 PERCENT OF STT NEURONS DOUBLE-LABELED: CONTRALATERAL CERVICAL ENLARGEMENT

%
(DL/SL&DL STT CELLS, n=NUMBER OF CASES WITH DL CELLS)

LAMINAE	(L-STT/MRF (N=6)	L-STT/PAG (N=5)	M-STT/MRF (N=6)	M-STT/PAG (N=5)
I	-	32% (60/187, n=5)	-	27% (11/41, n=4)
V	26% (14/53, n=3)	24% (16/67, n=5)	31% (20/64, n=6)	18% (9/51, n=2)
VI, VII, VIII	37% (15/40, n=4)	21% (6/28, n=3)	27% (21/79, n=6)	16% (11/68, n=3)
X	-	-	33% (3/9, n=2)	-
LSN	37% (9/24, n=2)	45% (29/65, n=4)	32% (7/22, n=2)	23% (26/112, n=5)

TABLE 14 PERCENT OF STT NEURONS DOUBLE-LABELED: IPSILATERAL CERVICAL ENLARGEMENT

% (DL/SL&DL STT CELLS, n = NUMBER OF CASES WITH DL CELLS)

LAMINAE	(L-STT/MRF (N=6)	L-STT/PAG (N=5)	M-STT/MRF (N=6)	M-STT/PAG (N=5)
I	-	25% (2/8,n=2)	-	-
V	9% (2/21, N=2)	20% (2/10, N=2)	35% (8/23, N=4)	15% (5/34, n=3)
VI, VII, VIII	-	-	29% (6/21, n=3)	-
X	-	-	-	-
LSN	-	55% (5/9, n=2)	15% (4/27, n=3)	-

contralateral and ipsilateral sides respectively. Both L-STT and M-STT neurons were double-labeled in somewhat higher percentages from the PAG or MRF in laminae V-VIII in the cervical as opposed to the lumbar segments. In lamina I, both L-STT and M-STT neurons were double-labeled from the PAG only. Double-labeled STT neurons were observed in the LSN in all four groups. On the ipsilateral side, double-labeled STT neurons were found in lamina V with both PAG and MRF injections. In contrast, no clear pattern of double-labeling was apparent in other ipsilateral laminae.

The pooled results of single- and double-labeled STT neurons in the cervical enlargement are illustrated in Figure 23. As seen in the data tables, about one third of the contralateral, lamina I STT cells were also labeled from the PAG. Also, there was a trend for more STT neurons to be double-labeled from the MRF than from the PAG in laminae VI-VIII; the reverse was true in the LSN. About the same proportion of STT neurons were double-labeled in lamina V in all four groups. Finally, there were no regions found to contain populations of STT neurons which were never double-labeled (like those seen in the VMDH).

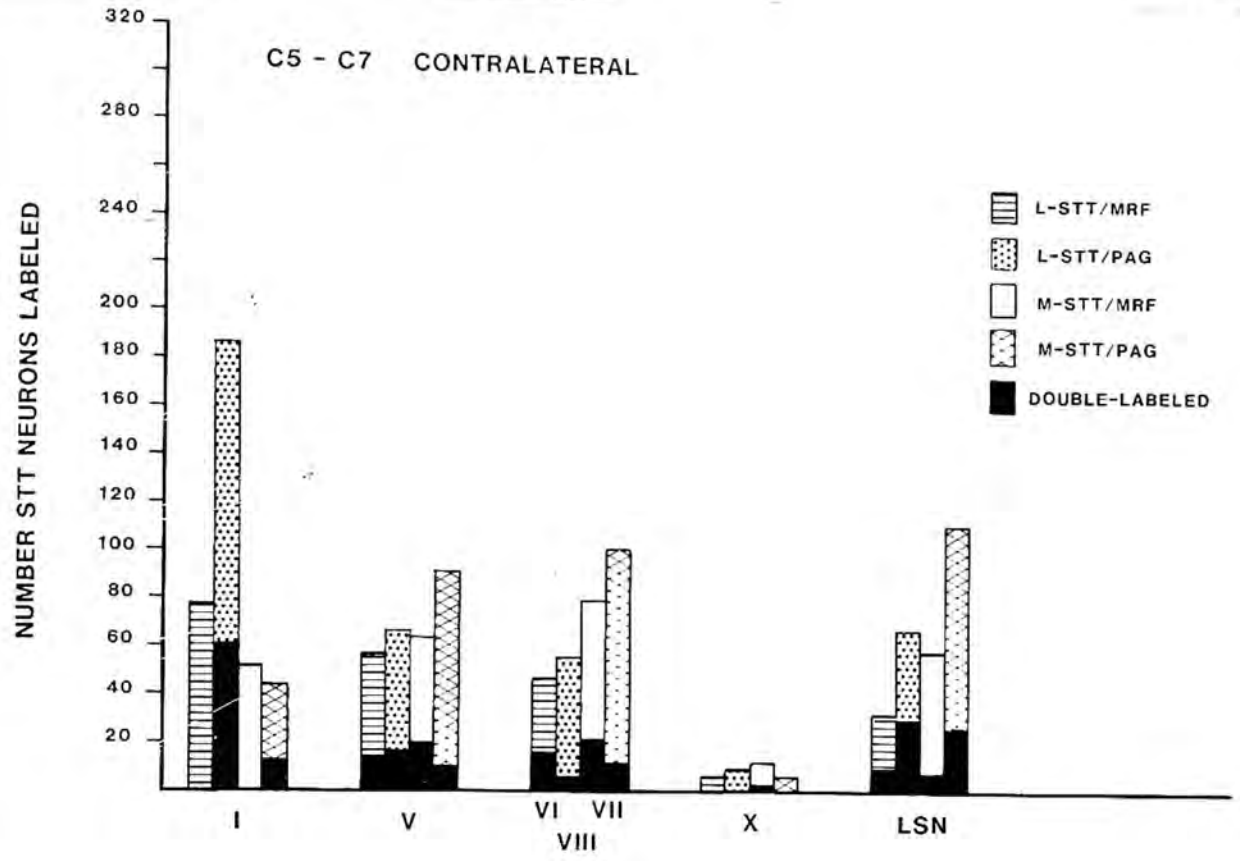
The data obtained in an L-STT/MRF case 136 are illustrated in Figure 24. In this case, double-labeled L-STT cells were located in laminae V-VIII. Many single-labeled , but no double-labeled, L-STT neurons were found in lamina I and in the LSN.

Figure 25 presents the findings in case 504, a L-

FIGURE 23

Histograms showing the total number of STT neurons in specific spinal laminae which were single- and double-labeled in each experimental group in the cervical enlargement. The data for each group are pooled from: 6 animals in the L-STT/MRF group; 5 in the L-STT/PAG group; 6 in the M-STT/MRF group; and 5 in the M-STT/PAG group.

FIGURE 23


C5 - C7 CONTRALATERAL
NUMBER STT NEURONS LABELED
320
280
240
200
180
160
140
120
100
80
60
40
20
L-STT/MRF
L-STT/PAG
M-STT/MRF
M-STT/PAG
DOUBLE-LABELED
I
V
VI VII
VIII
X
LSN

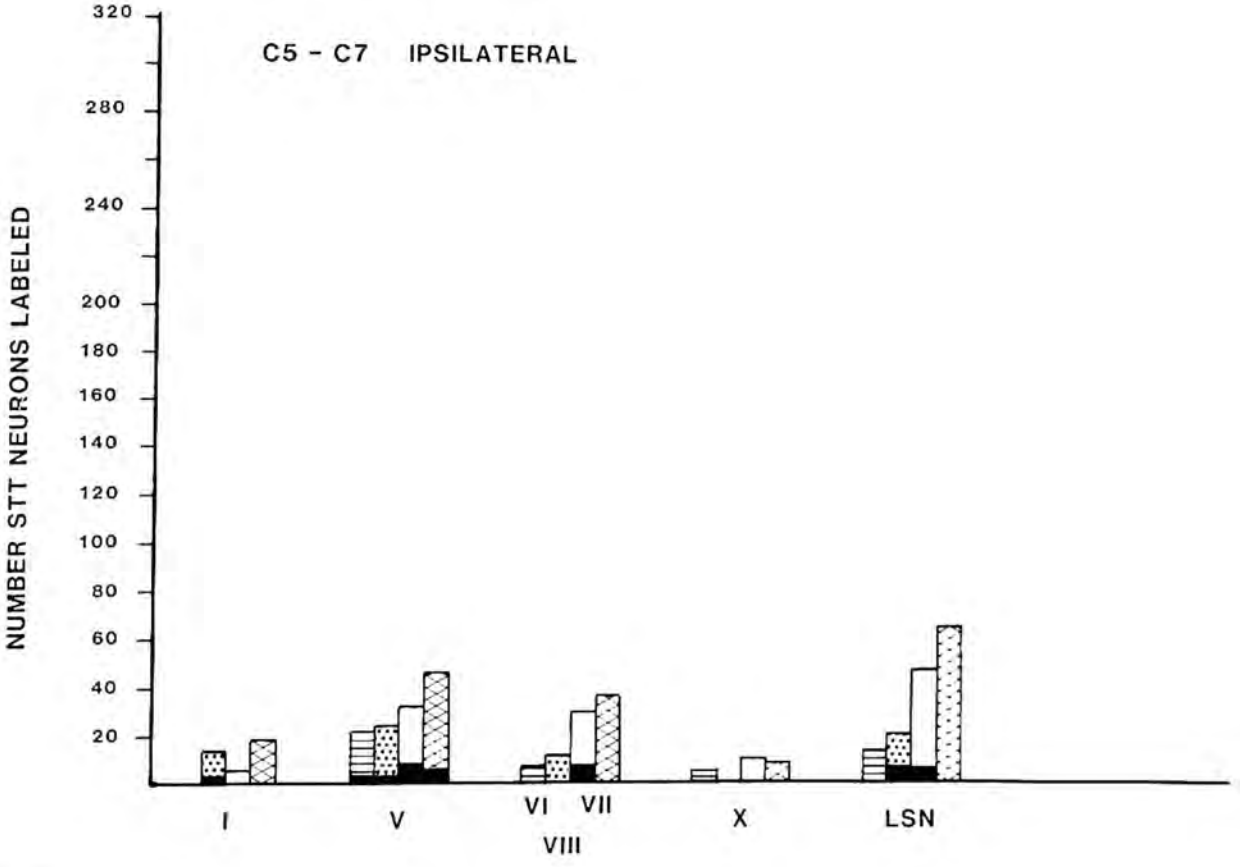

C5 - C7 IPSILATERAL
NUMBER STT NEURONS LABELED
320
280
240
200
180
160
140
120
100
80
60
40
20
I
V
VI VII
VIII
X
LSN

FIGURE 24

Line drawings illustrating the number and locations of single and double-labeled neurons in the cervical enlargement of case 136, a L-STT/MRF case. Labeled L-STT neurons are shown in the top drawing and neurons labeled from the MRF are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

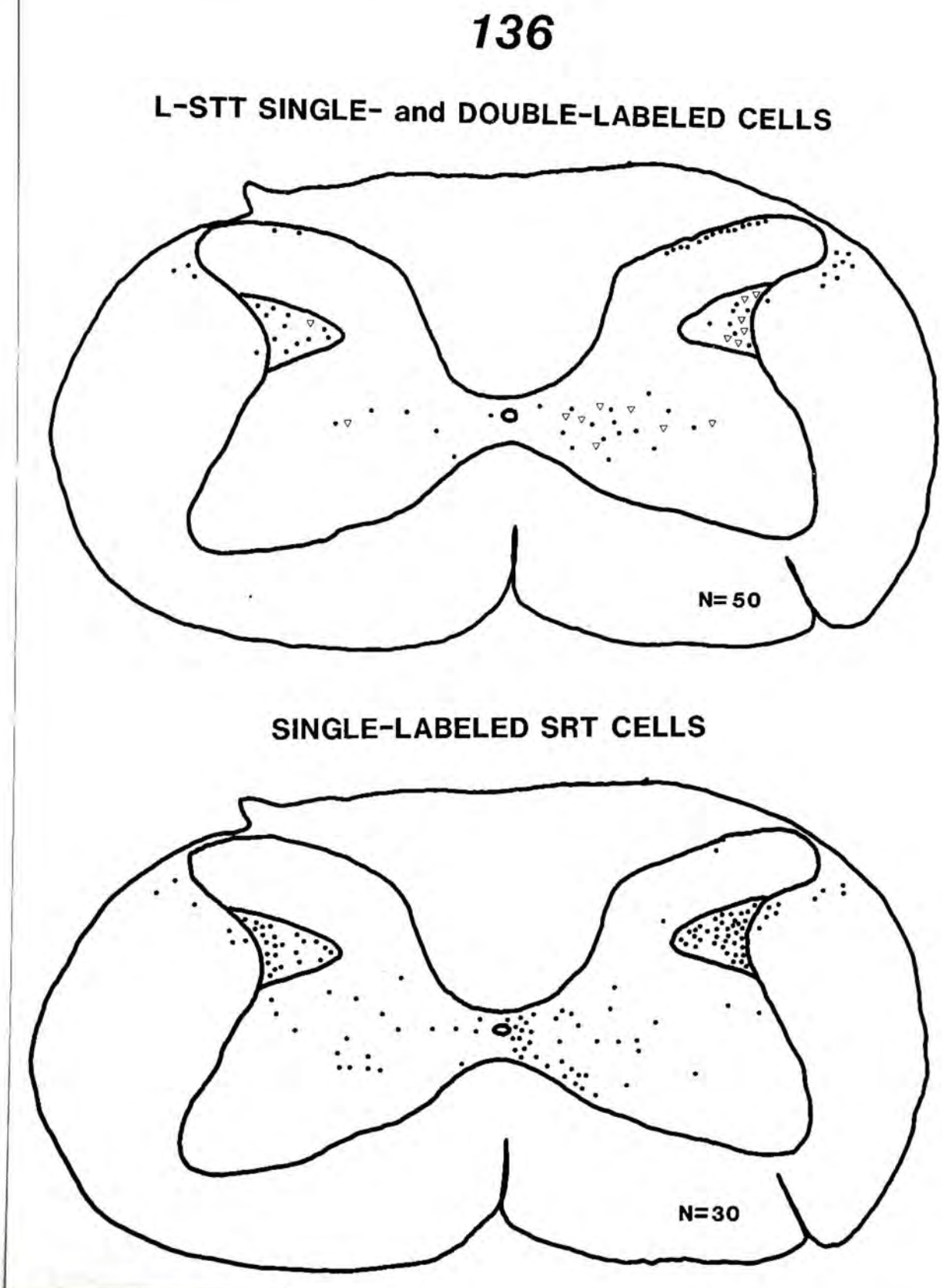
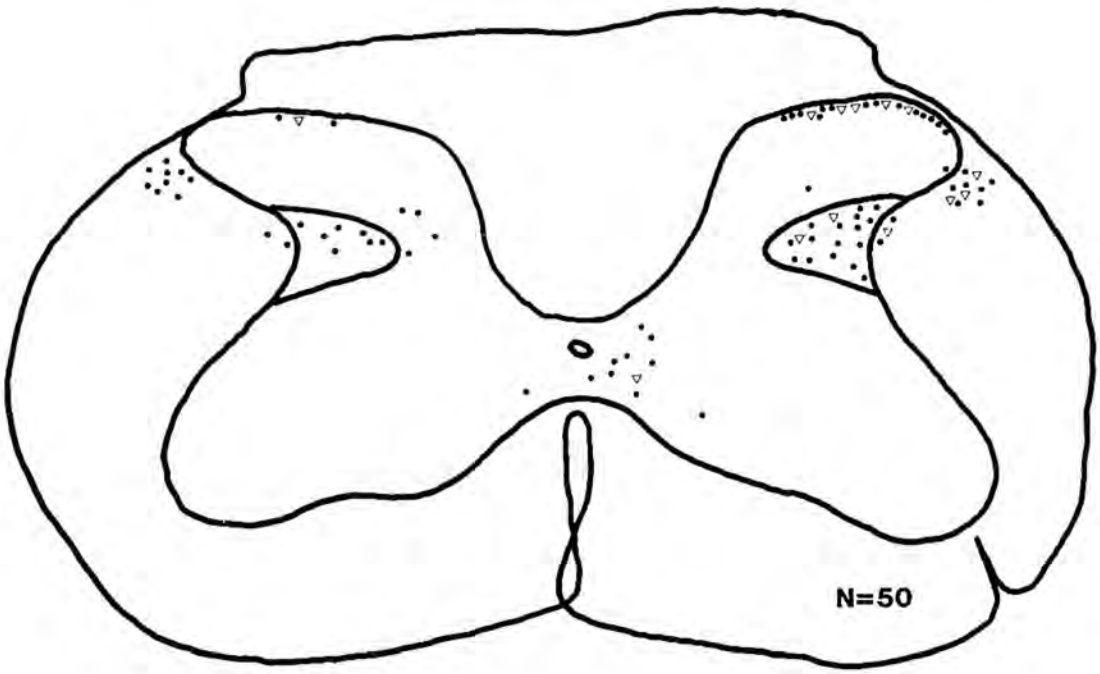

136
L-STT SINGLE- and DOUBLE-LABELED CELLS
N=50
SINGLE-LABELED SRT CELLS
N=30

FIGURE 25

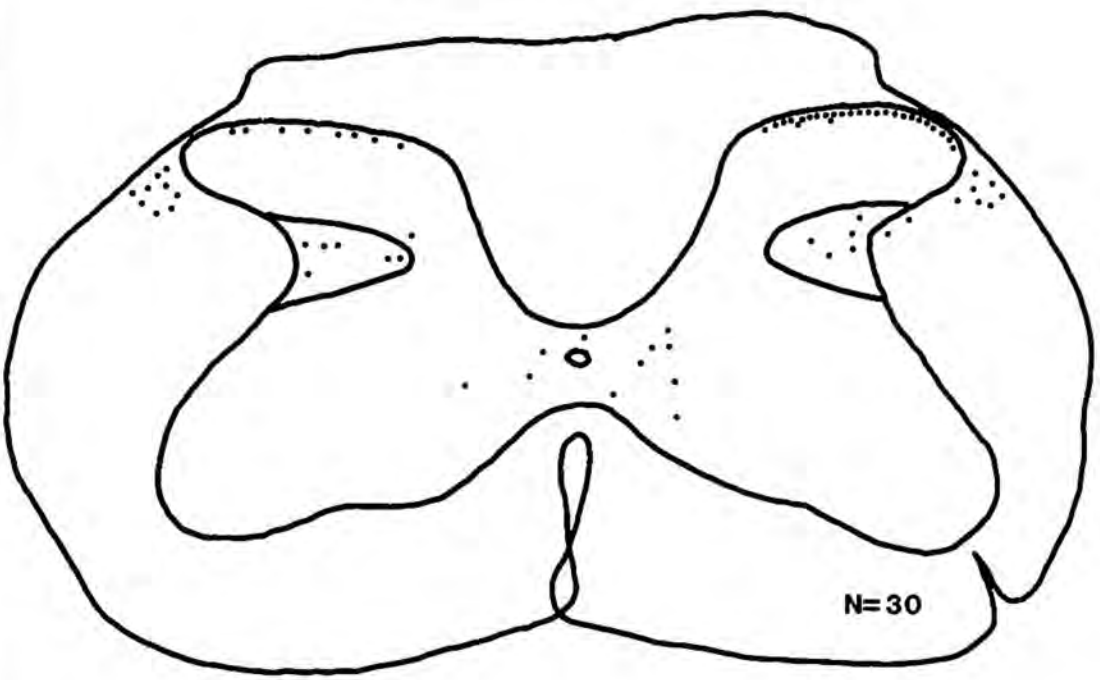
Line drawings illustrating the number and locations of single and double-labeled neurons in the cervical enlargement of case 504, a L-STT/PAG case. Labeled L-STT neurons are shown in the top drawing and neurons labeled from the PAG are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

504

L-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SAT CELLS



STT/PAG animal. Both lamina I and the LSN contained double-labeled neurons, consistent with the pooled data for this group. There were also neurons containing FB and RhS in lamina V and one such cell on the lamina VII-VIII border.

No double-labeled and only two single-labeled M-STT neurons were observed in lamina I in case 129 (M-STT/MRF), which is illustrated in Figure 26. M-STT neurons double-labeled from the MRF were restricted to the contralateral laminae V and VII. Only single-labeled M-STT cells were found in the LSN in this case.

In the M-STT/PAG case (303), shown in Figure 27, there were many neurons in lamina I single-labeled from the PAG, but only two single-labeled M-STT cells were observed. In general, cervical enlargement labeling was sparse in this animal. On the contralateral side, there was a double-labeled cell in the LSN, one in lamina VII and one in lamina VIII. Two such M-STT neurons were found ipsilaterally (in the LSN and lamina V).

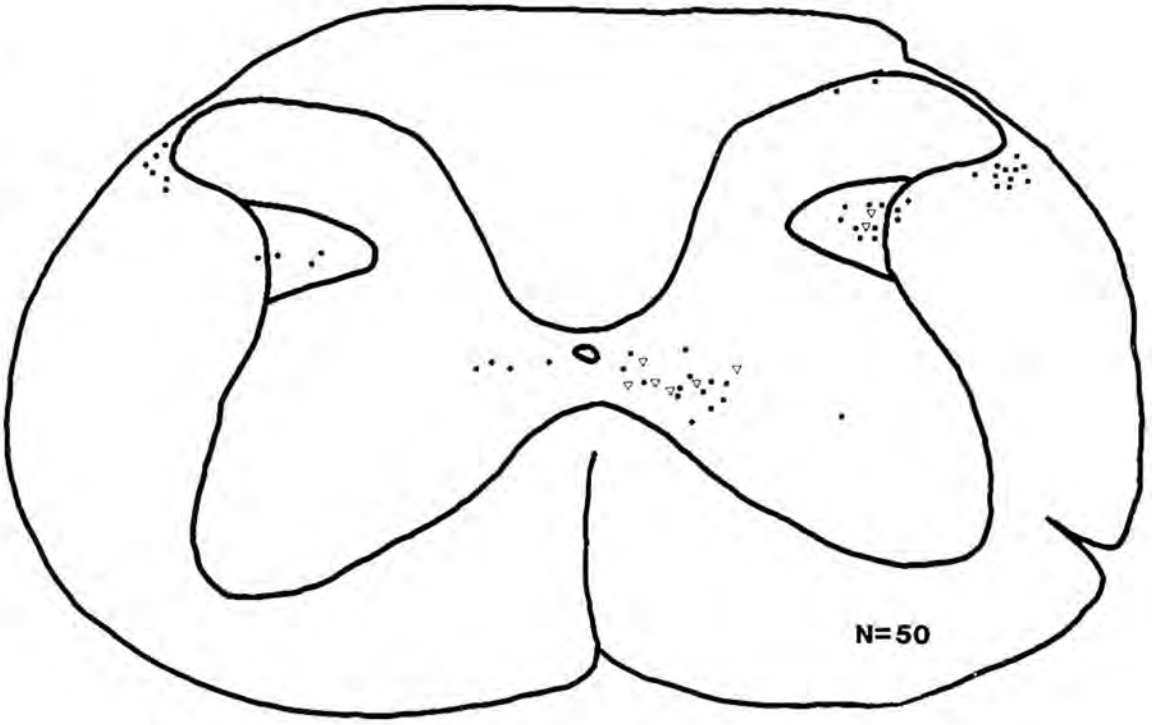
In summary, in the cervical enlargement, both L-STT and M-STT neurons were double-labeled from the MRF or PAG in the contralateral laminae V-VIII and in the LSN. A larger percentage of STT neurons were double-labeled in the LSN following PAG injections than following MRF injections. In laminae VI-VIII, more STT cells were double-labeled from the MRF than were double-labeled from the PAG. Lamina I contained mostly L-STT neurons; these,as well as M-STT cells, were double-labeled only from the PAG. Finally,

FIGURE 26

Line drawings illustrating the number and locations of single and double-labeled neurons in the cervical enlargement of case 129, a M-STT/MRF case. Labeled M-STT neurons are shown in the top drawing and neurons labeled from the MRF are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The large dots represent 10 single-labeled neurons. The right side of each drawing is contralateral to the injection sites.

129

M-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SRT CELLS

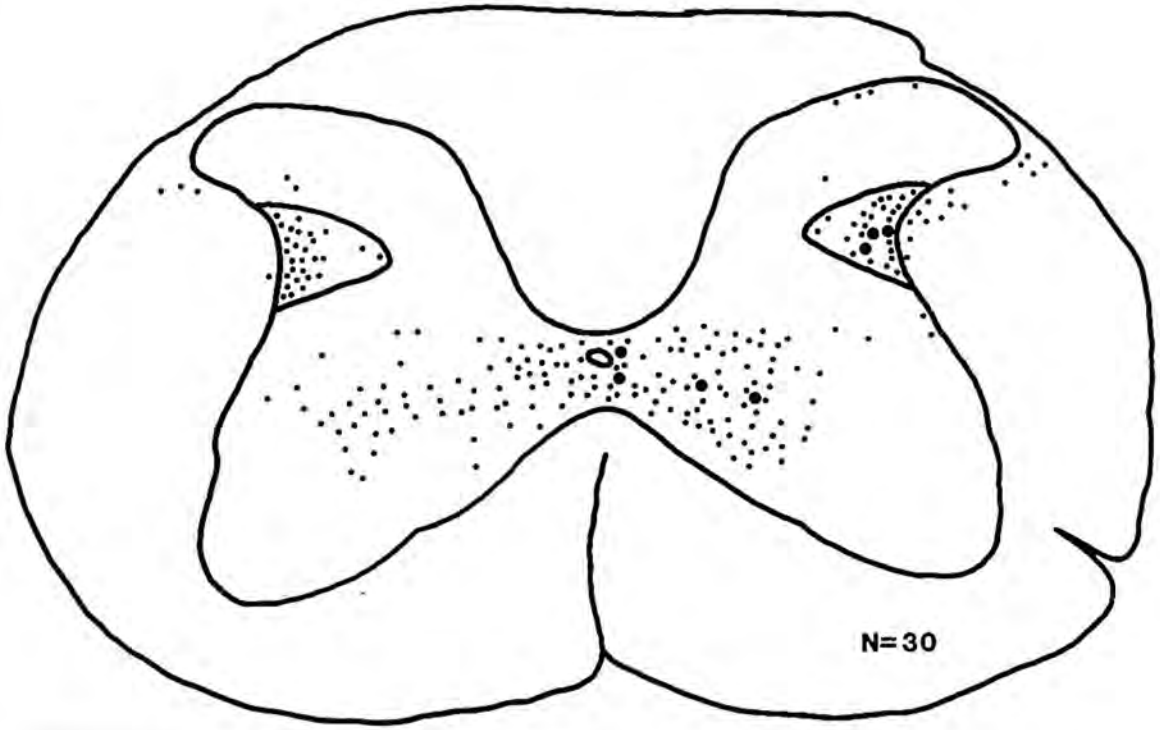
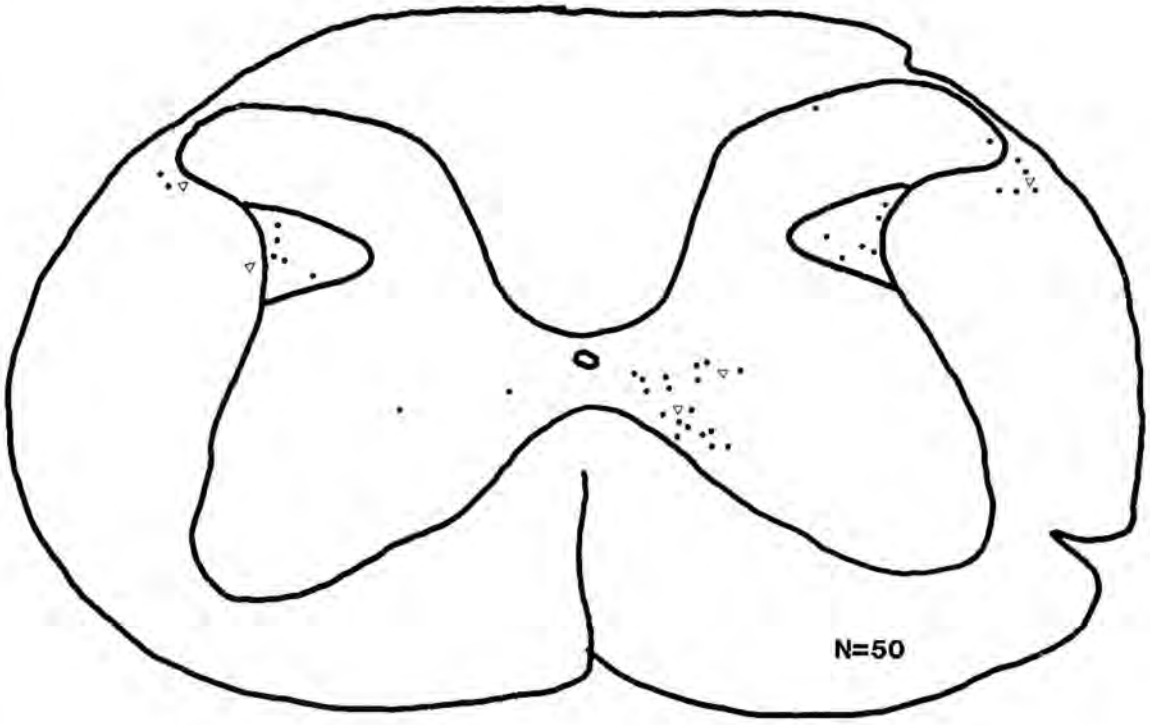


FIGURE 27

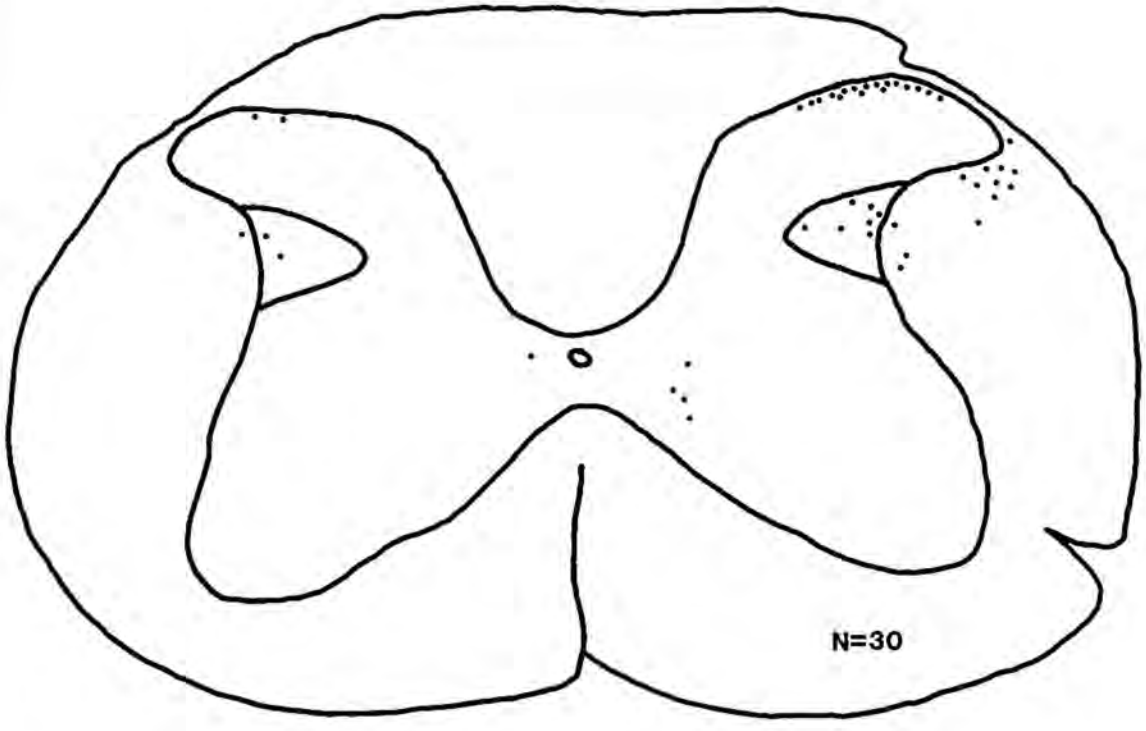
Line drawings illustrating the number and locations of single- and double-labeled neurons in the cervical enlargement of case 303, a M-STT/PAG case. Labeled M-STT neurons are shown in the top drawing and neurons labeled from the PAG are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

303

M-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SAT CELLS



unlike the lumbar enlargement, no area of the cervical enlargement contained exclusively single-labeled STT neurons of either type. Examples of double-labeled STT neurons in laminae I, V, and the LSN are presented in the photomicrographs in Figure 28.

Mid-Thoracic Segments

The laminar boundaries used for mid-thoracic segments were from Paxinos and Watson's atlas (1982) and are illustrated in Figure 29. Generally, cell labeling in these segments was sparse, especially on the ipsilateral side. The distribution of L-STT and M-STT labeled neurons is presented in Table 15. With the exception that there was more bilateral labeling in M-STT groups, little difference was observed in the number or location of L-STT versus M-STT neurons. Both types of cells were common in laminae V,VII and VIII, and were present, but sparse, in laminae I,X, and the LSN.

The average numbers of spinoreticular and spinoannular tract neurons are presented in Table 16. Far more neurons were labeled from the MRF than from the PAG and most of these were located bilaterally, with contralateral predominance, in laminae V,VII and VIII. There were a few neurons labeled from the MRF and the PAG in lamina X and the LSN of both sides. Lamina I contained only spinoannular tract cells.

The number and percentages of double-labeled STT

FIGURE 28

Photomicrographs of three double-labeled neurons in the cervical enlargement (742X). A cell found contralaterally in lamina V in case 140 (M-STT/MRF) is shown in A and B. This neuron contained FB (A) and RhS (B). A double-labeled lamina I neuron containing FB and RhS is shown in C and D respectively. This cell was found in a L-STT/PAG case (502). Finally, a double-labeled neuron in the contralateral lateral spinal nucleus of case 502 is presented in E (FB) and F (RhS). These photomicrographs were taken under either 420 nm (A,C,E) or 550 nm (B,D,F) wavelength light.

FIGURE 28

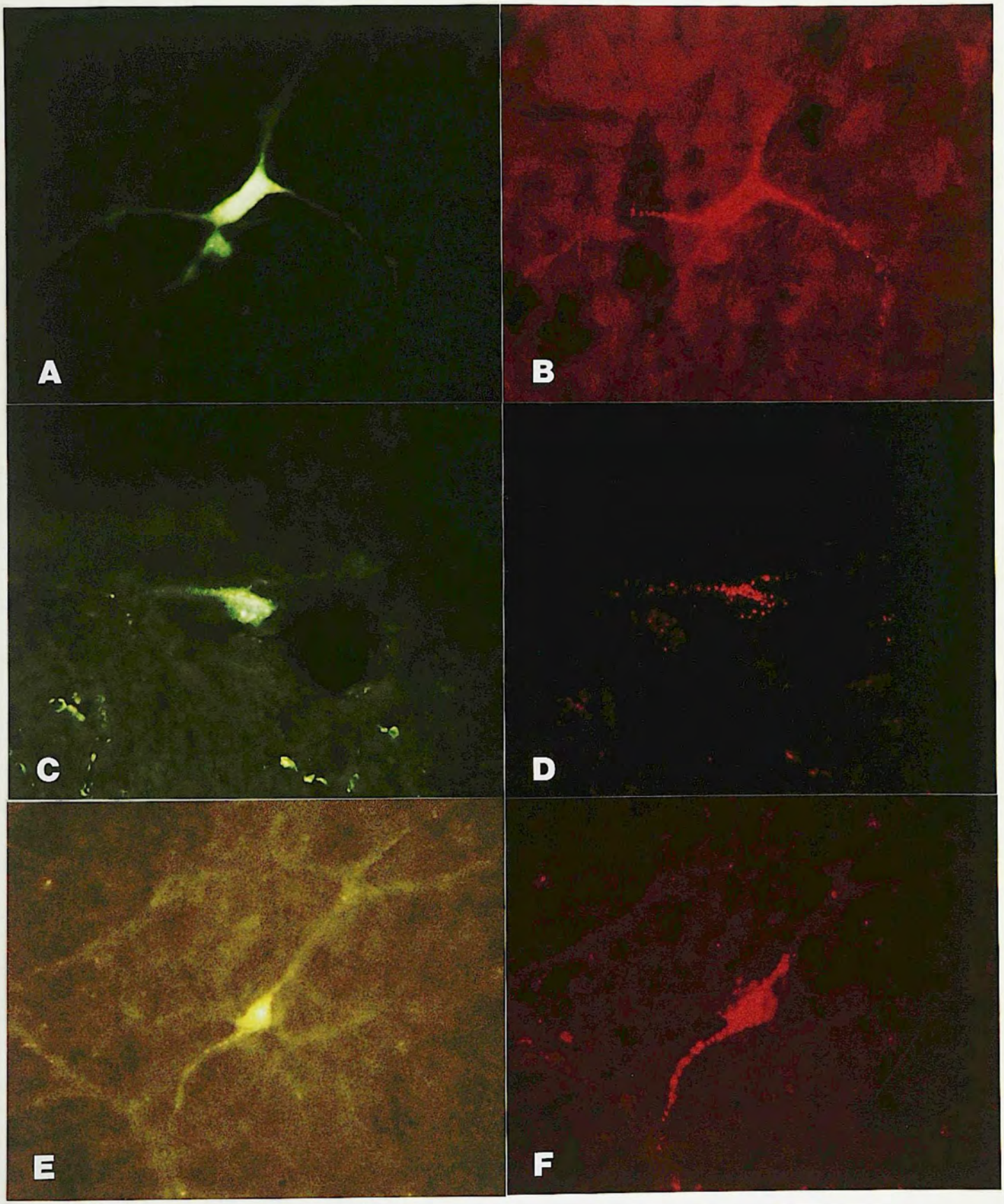


FIGURE 29

Line drawing of a transverse spinal section illustrating the laminar boundaries used in the present study for the mid-thoracic segments. Note that there is no lamina VI at these spinal levels.

FIGURE 29

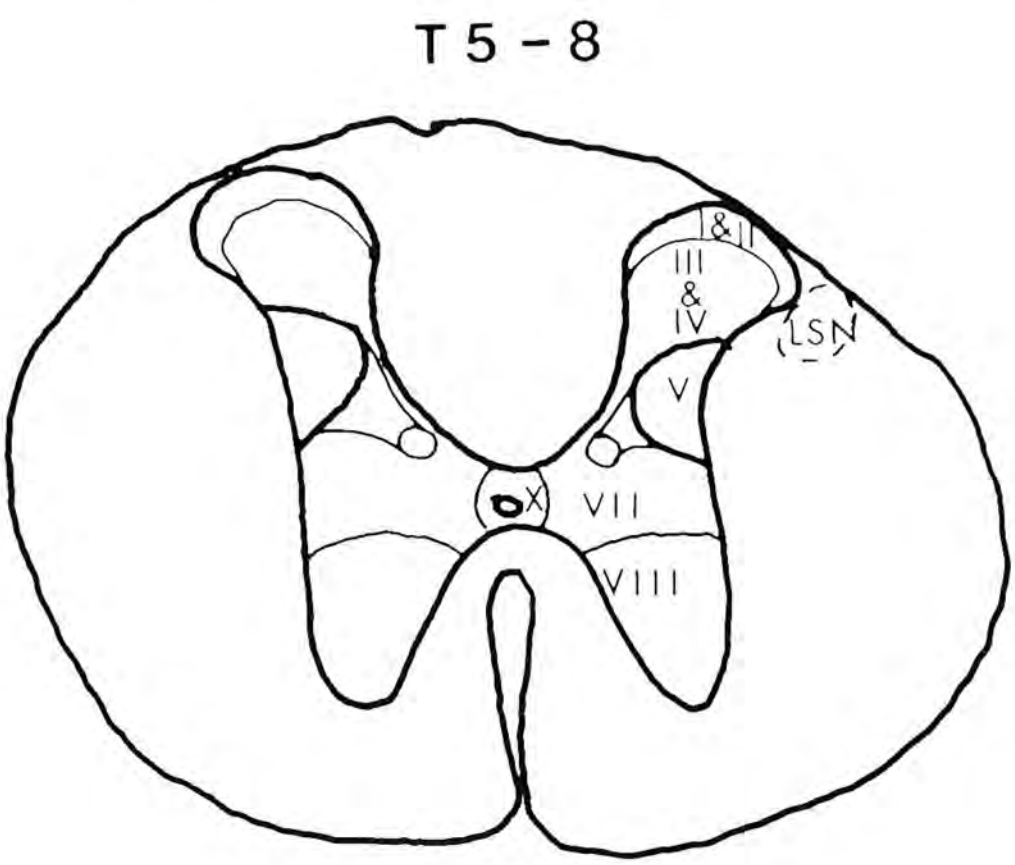


TABLE 15

NUMBER OF STT NEURONS LABELED: MID-THORACIC SEGMENTS

$\bar{X} \pm$ S.E.M., Number labeled in 50 (25μm) sections from each case

LAMINAE	L-STT (N=11)	M-STT (N=11)
CONTRALATERAL		
I	1.7 ± 0.7	1.0 ± 0.5
V	7.8 ± 2.0	9.5 ± 1.2
VII, VIII	12.2 ± 3.0	11.4 ± 1.8
X	2.7 ± 0.6	2.0 ± 0.8
LSN	4.0 ± 1.8	4.7 ± 0.9
IPSILATERAL		
I	-	0.3 ± 0.2
V	1.4 ± 0.6	3.3 ± 0.9
VII, VIII	1.0 ± 0.4	2.4 ± 0.8
X	-	1.0 ± 0.3
LSN	2.1 ± 0.6	4.3 ± 0.9

TABLE 16

NUMBER OF NEURONS LABELED FROM THE PAG OR MRF: MID-THORACIC SEGMENTS

$\bar{X} \pm$ S.E.M., Number labeled in 30 (25μm) sections from each case

LAMINAE	SRT (N=12)	SAT (N=10)
CONTRALATERAL		
I	-	3.9 ± 0.8
V	20.7 ± 4.3	4.9 ± 1.4
VII, VIII	39.5 ± 5.8	4.8 ± 1.1
X	8.7 ± 1.7	3.4 ± 0.8
LSN	6.3 ± 2.1	6.2 ± 1.2
IPSILATERAL		
I	-	1.2 ± 0.2
V	19.3 ± 3.6	2.5 ± 0.7
VII, VIII	17.5 ± 4.3	1.1 ± 0.4
X	5.4 ± 1.5	2.3 ± 0.3
LSN	5.6 ± 1.1	3.5 ± 0.9

neurons that were observed contralateral and ipsilateral to the injection sites are shown in Table 17 and Table 18. The majority of double-labeled neurons were present in the contralateral laminae V,VII and VIII. A few were found in lamina I in the L-STT/PAG group and in lamina X in the L-STT/MRF group. L-STT and M-STT neurons in the LSN were only found to be double-labeled in cases with tracer injections into the PAG. Ipsilaterally, double-labeled neurons were observed in laminae VII and VIII and in the LSN. However, very few cells were labeled and, thus, the variability was quite high.

Figure 30 presents the total number of STT neurons, single- and double-labeled, observed in each of the four groups. Due to the paucity of ipsilateral labeling, only the contralateral cell counts are included in this histogram. Both L-STT and M-STT neurons were double-labeled from the MRF or PAG in laminae V,VII and VIII. STT neurons in the LSN often contained a second label of RhS following PAG, but not MRF, injections.

Data from each of the individual cases, 136,504,129, and 303, are illustrated in Figures 31, 32, 33, and 34 respectively. These individual cases conform well to the group data in that double-labeled M-STT and L-STTT neurons were found in all four cases in lamina VII, and, in three out of four cases, in lamina V. Photomicrographs of some double-labeled STT neurons in the mid-thoracic spinal cord are presented in Figure 35.

TABLE 17 PERCENT OF STT NEURONS DOUBLE-LABELED: CONTRALATERAL MID-THORACIC SEGMENTS

% (DL/SL&DL STT CELLS, n=NUMBER OF CASES WITH DL CELLS)

LAMINAE	L-STT/MRF (N=6)	L-STT/PAG (N=5)	M-STT/MRF (N=6)	M-STT/PAG (N=5)
I	-	33% (3/10, n=2)	-	-
V	30% (10/33, n=3)	21% (6/29, n=3)	23% (11/48, n=6)	7% (3/44, n=3)
VII, VIII	22% (14/63, n=4)	14% (10/71, n=5)	40% (19/48, n=5)	9% (5/54, n=4)
X	27% (3/11, n=2)	-	-	-
LSN	-	41% (13/32, n=2)	-	24% (6/25, n=3)

TABLE 18 PERCENT OF STT NEURONS DOUBLE-LABELED: IPSILATERAL MID-THORACIC SEGMENTS

% (DL/SL&DL STT CELLS, n = NUMBER OF CASES WITH DL CELLS)

LAMINAE	L-STT/MRF (N=6)	L-STT/PAG (N=5)	M-STT/MRF (N=6)	M-STT/PAG (N=5)
I	-	-	-	-
V	-	-	-	-
VII, VIII	-	33% (2/6, N=2)	22% (2/9, N=2)	-
X	-	-	-	-
LSN	-	25% (3/12, N=2)	33% (3/10, N=3)	20% (3/15, n=2)

FIGURE 30

Histogram showing the total number of STT neurons in specific, contralateral, spinal laminae which were single- and double-labeled in each experimental group in the mid-thoracic segments. The data for each group are pooled from: 6 animals in the L-STT/MRF group; 5 in the L-STT/PAG group; 6 in the M-STT/MRF group; and 5 in the M-STT/PAG group. Due to the paucity of ipsilateral labeling in mid-thoracic segments, only the contralateral data are shown.

FIGURE 30

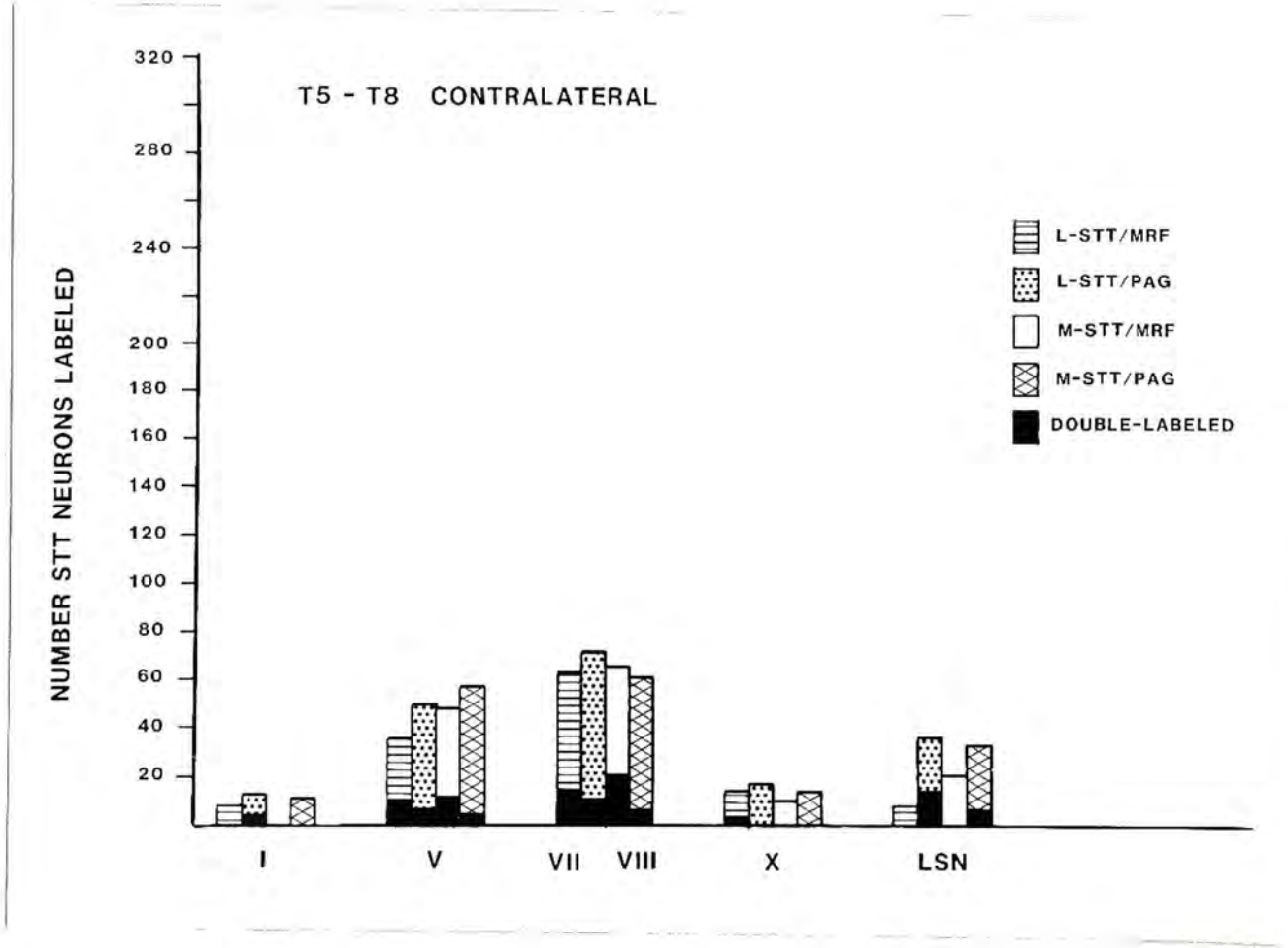

T5 - T8 CONTRALATERAL
NUMBER STT NEURONS LABELED
320
280
240
200
180
160
140
120
100
80
60
40
20
L-STT/MRF
L-STT/PAG
M-STT/MRF
M-STT/PAG
DOUBLE-LABELED
I
V
VII
VIII
X
LSN

FIGURE 31

Line drawings illustrating the number and locations of single- and double-labeled neurons in the mid-thoracic spinal cord of case 136, a L-STT/MRF case. Labeled L-STT neurons are shown in the top drawing and neurons labeled from the MRF are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

FIGURE 31

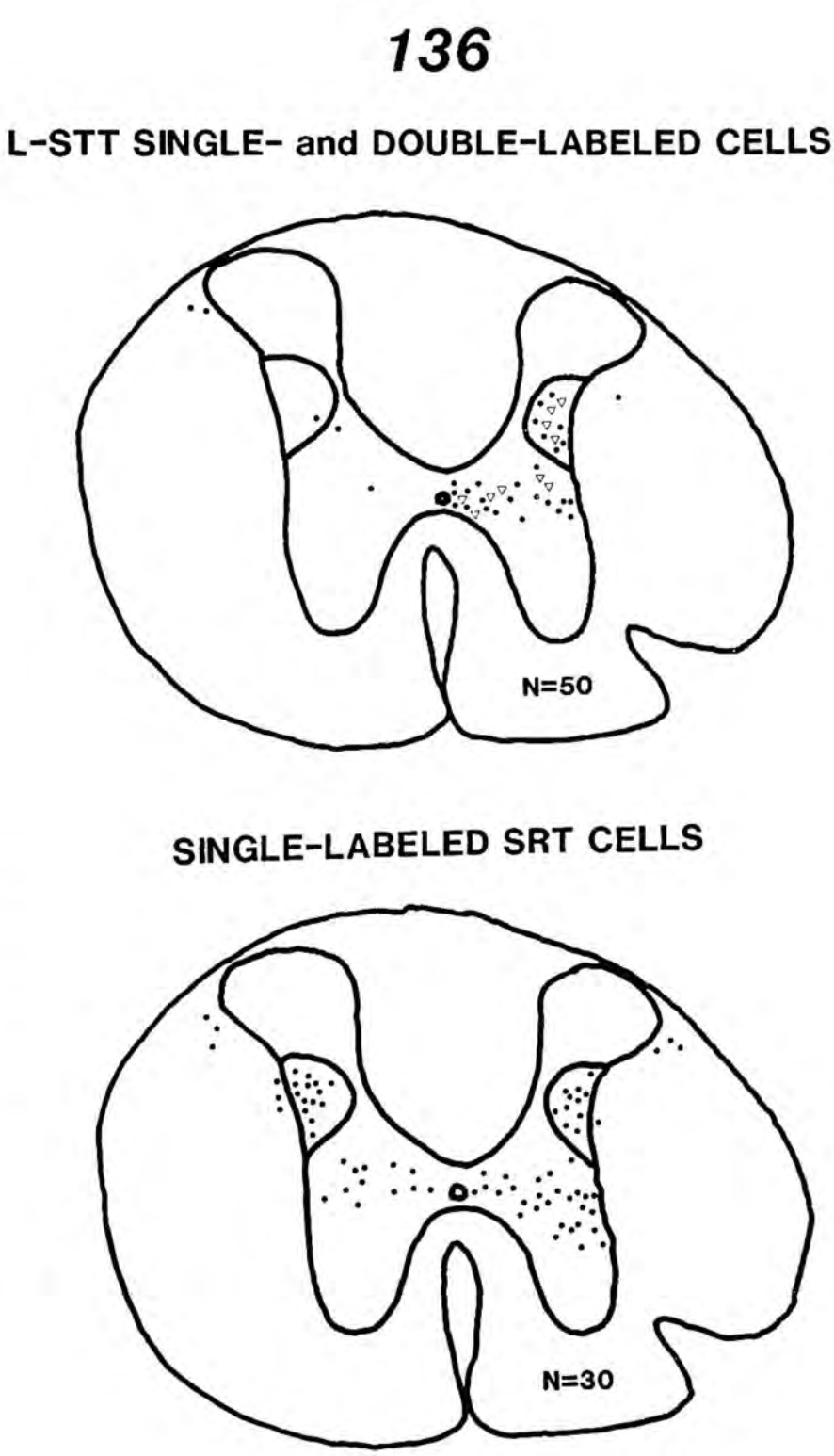

136
L-STT SINGLE- and DOUBLE-LABELED CELLS
N=50
SINGLE-LABELED SRT CELLS
N=30

FIGURE 32

Line drawings illustrating the number and locations of single- and double-labeled neurons in the mid-thoracic spinal cord of case 504, a L-STT/PAG case. Labeled L-STT neurons are shown in the top drawing and neurons labeled from the PAG are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

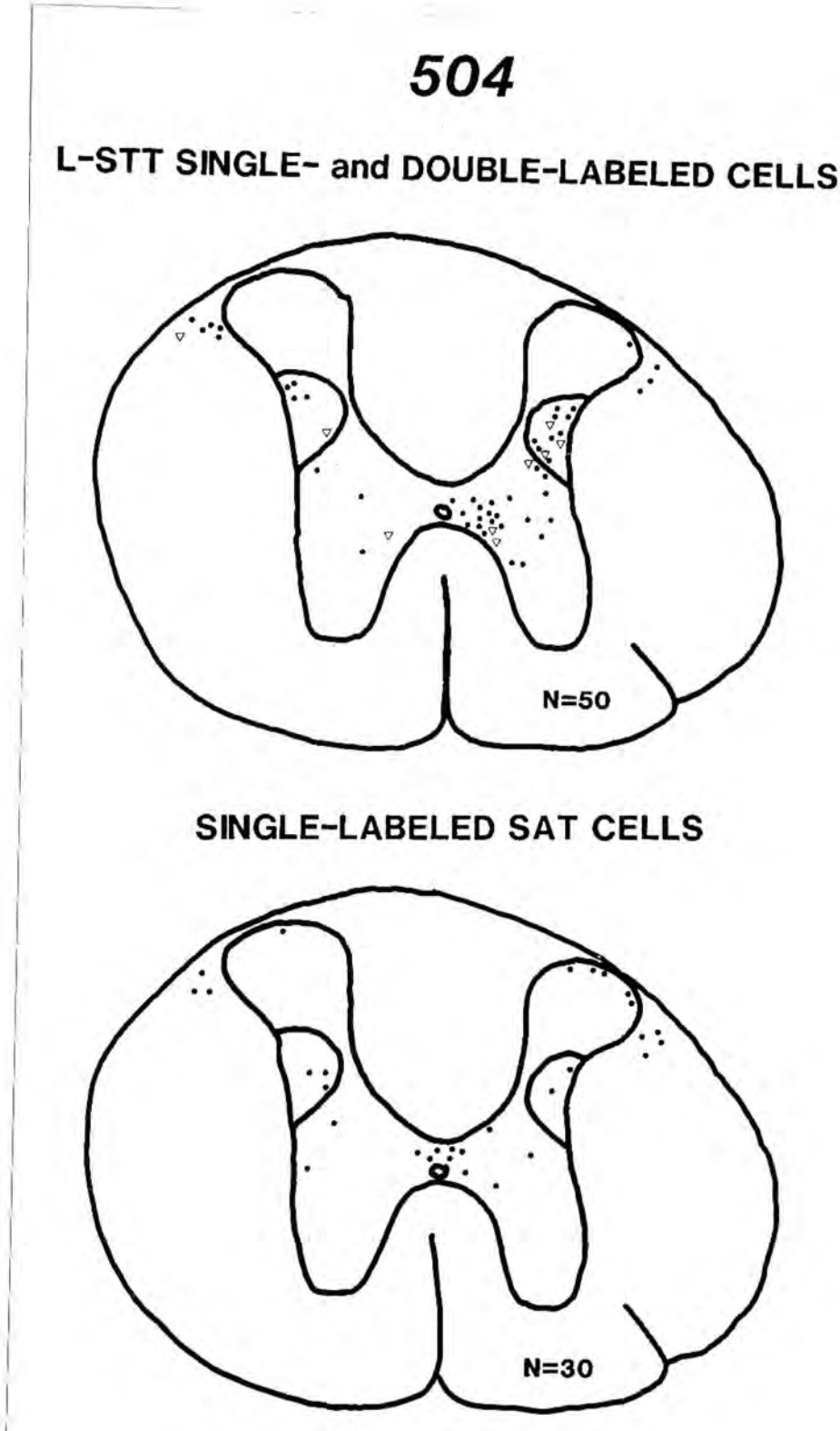
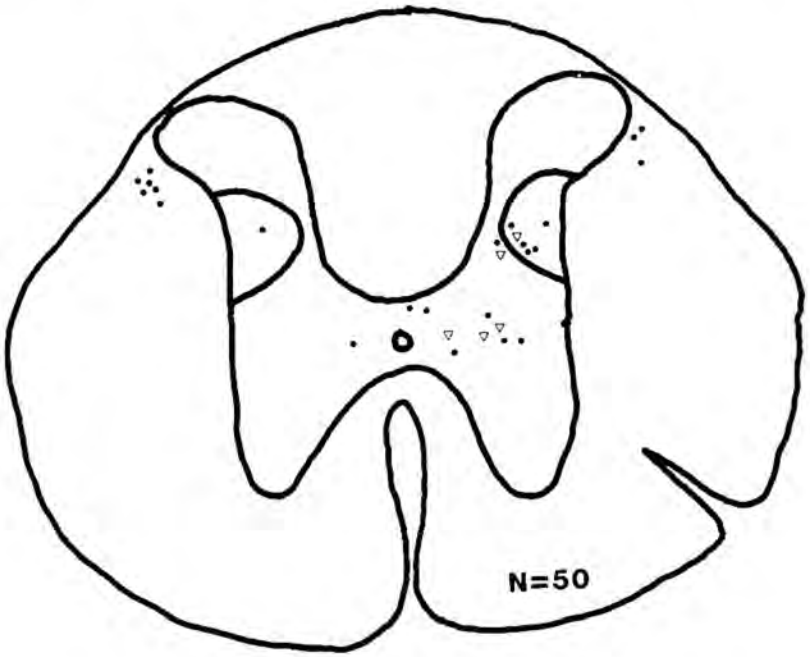

504
L-STT SINGLE- and DOUBLE-LABELED CELLS
N=50
SINGLE-LABELED SAT CELLS
N=30

FIGURE 33

Line drawings illustrating the number and locations of single- and double-labeled neurons in the mid-thoracic spinal cord of case 129, a M-STT/MRF case. Labeled M-STT neurons are shown in the top drawing and neurons labeled from the MRF are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

129

M-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SRT CELLS

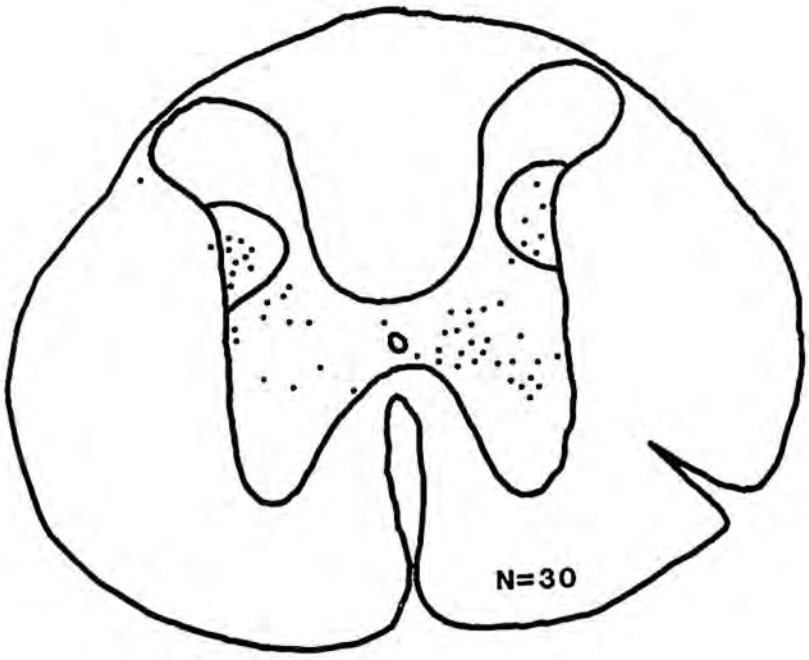
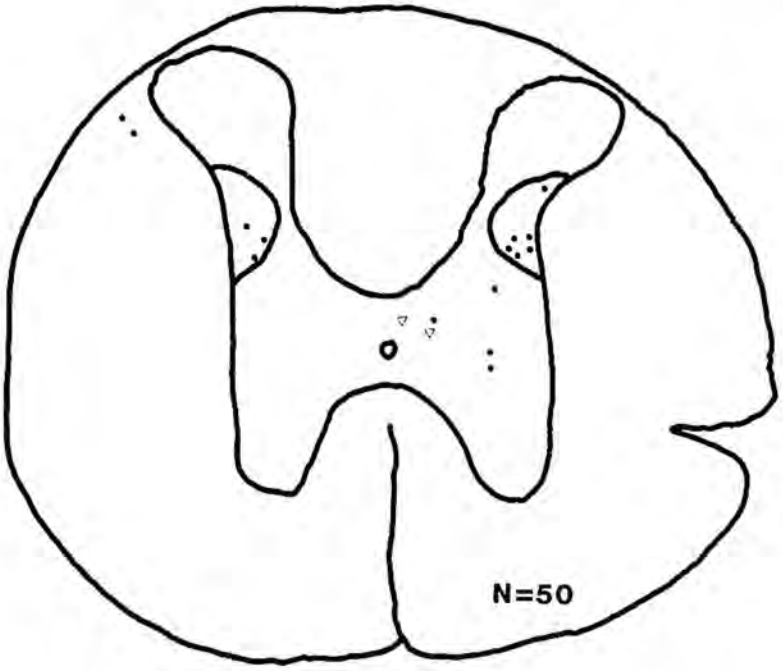


FIGURE 34

Line drawings illustrating the number and locations of single- and double-labeled neurons in the mid-thoracic spinal cord of case 303, a M-STT/PAG case. Labeled M-STT neurons are shown in the top drawing and neurons labeled from the PAG are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The right side of each drawing is contralateral to the injection sites.

303

M-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SAT CELLS

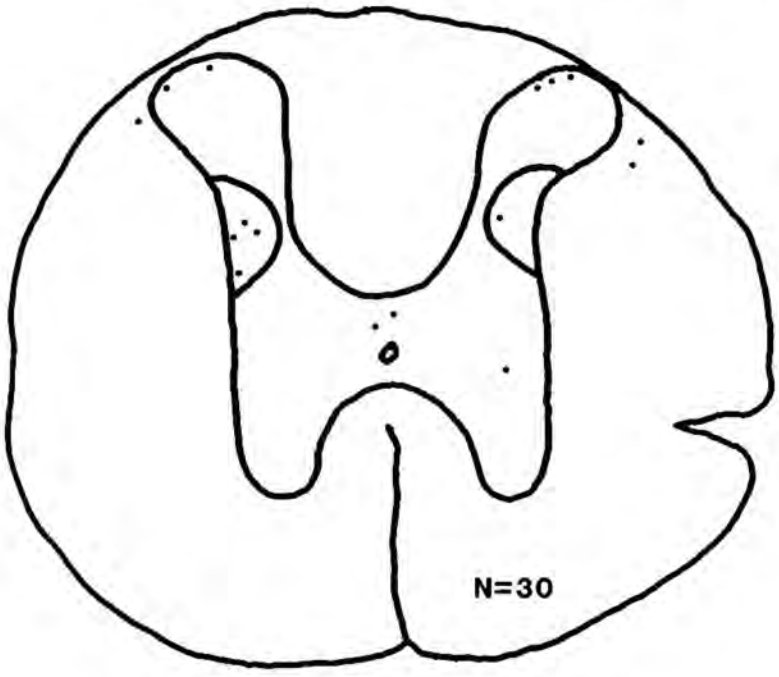
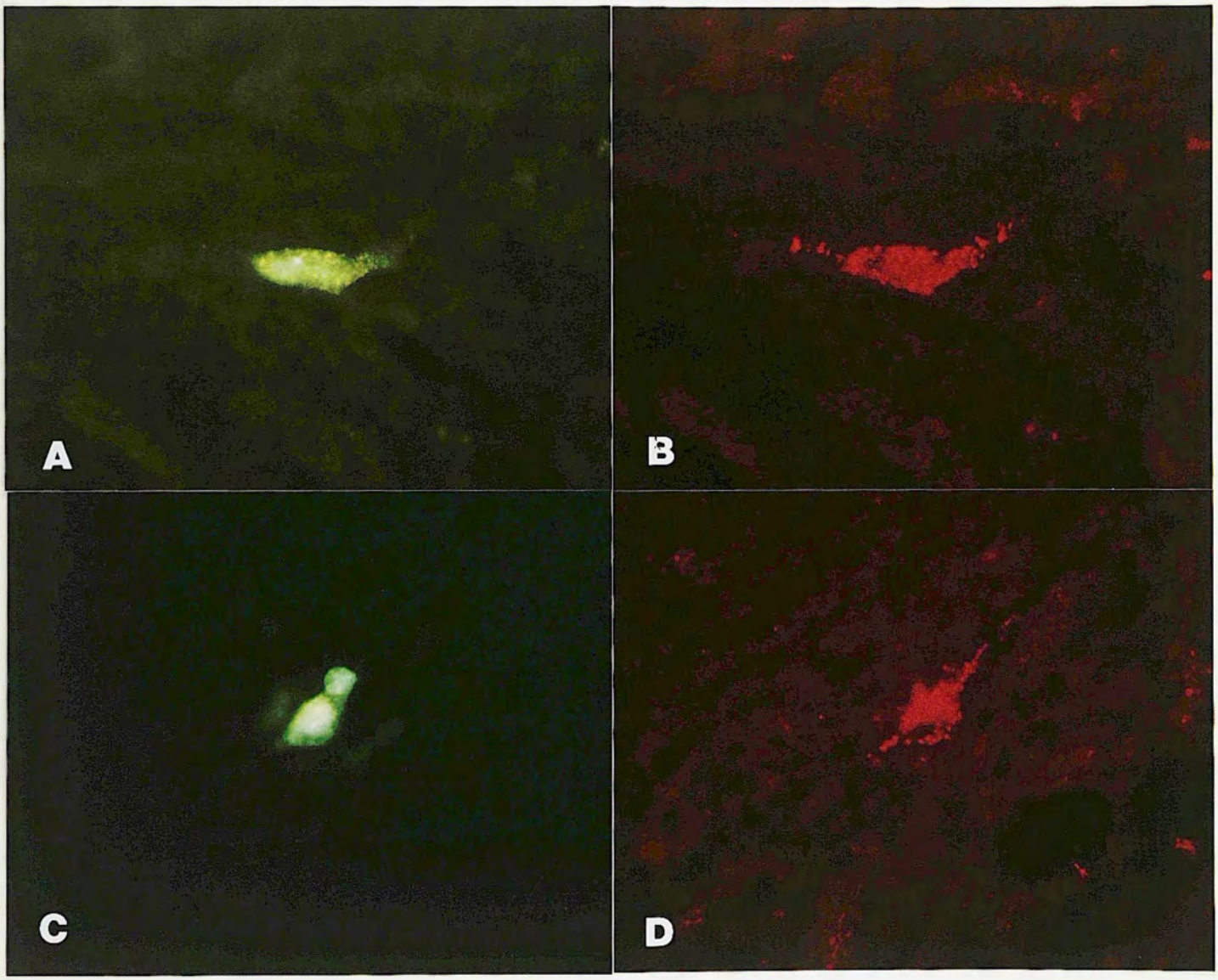


FIGURE 35

Photomicrographs (742X) of two double-labeled neurons found in mid-thoracic segments. Both neurons contained FB (A,C) and RhS (B,D). The cell in A and B was found in lamina X, contralateral to the injection sites, in case 124 (L-STT/MRF). In C and D is shown a neuron which was observed in case 142 (M-STT/MRF) in contralateral lamina V.

FIGURE 35



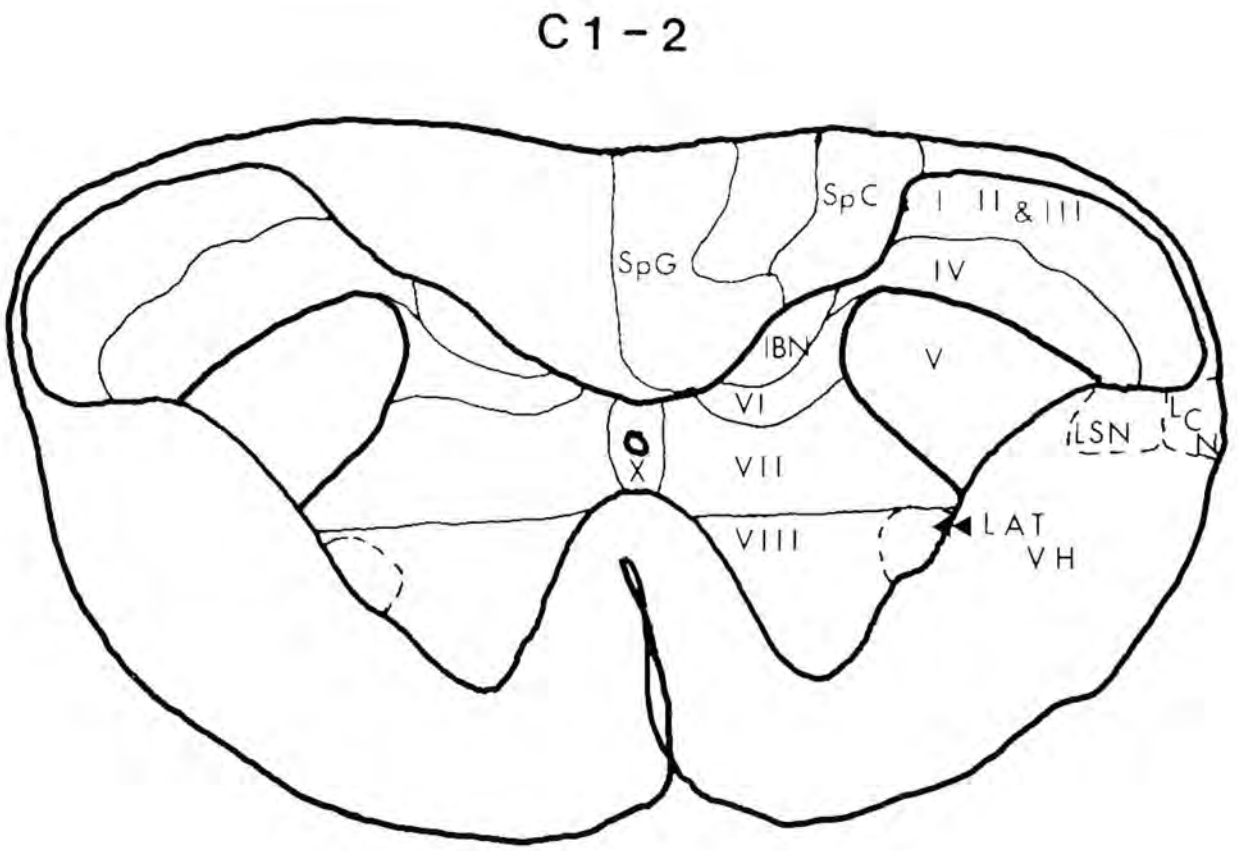
Upper Cervical Segments

The greatest number of STT neurons, as well as neurons labeled from the MRF and PAG, was observed in the upper cervical segments of the spinal cord. Figure 36 illustrates the laminar delineations used in these studies. The lateral cervical nucleus and the LSN are both present in the upper cervical segments as described by Giesler and Elde (1985). Laminae I-V correspond to Rexed's (1952) description in the cat. The internal basilar nucleus, described by Torvik (1956), occupies the ventromedial part of the dorsal horn. Surrounding this nucleus is lamina VI. Rexed regarded the internal basilar nucleus as the medial part of lamina VI which differed from the lateral portion in that it contained larger, more densely packed, neurons. Paxinos and Watson defined this region as the ventromedial extension of lamina IV. However, in addition to the different cytoarchitecture described by Rexed, fiber bundles which course between the internal basilar nucleus and lamina VI provide a natural boundary separating the two areas. Finally, the internal basilar nucleus is described separately due to the present observation of distinct differences in labeling in this nucleus compared to lamina VI. Laminae VII, VIII and X conform to both Rexed's (1952) and Paxinos and Watson's (1982) delineations, as do the boundaries of the spinal extensions of the dorsal column nuclei. However, an additional area, the lateral ventral horn, is outlined in

FIGURE 36

Line drawing of a transverse spinal section illustrating the laminar boundaries used in the present study for the upper cervical segments.

FIGURE 36



the most lateral part of lamina VIII. This region, not equivalent to the motor nucleus of the spinal accessory nerve, is included for emphasis since it also pertains to the present data.

The mean number of STT neurons found in specific laminae of the upper cervical spinal cords is detailed in Table 19. Generally, fewer labeled cells were seen in the M-STT group than in the L-STT group. There were a few L-STT and M-STT cells in lamina I, mostly on the contralateral side. Lamina V and VI contained many STT neurons of both types in a mainly contralateral distribution. In contrast, bilateral labeling, with ipsilateral predominance, was observed for L-STT and M-STT groups in laminae VII and VIII. Lamina X contained very few STT neurons and the LSN contained moderate numbers of both L-STT and M-STT cells, most of which were contralaterally located.

Clear differences between L-STT and M-STT groups were seen in the internal basilar and lateral cervical nuclei. In these regions, many neurons were labeled from the lateral thalamus in an almost entirely contralateral distribution; whereas very few labeled cells in either of the two nuclei were seen in the M-STT groups.

The number of upper cervical neurons labeled from the MRF or PAG is presented in Table 20. As observed in other spinal segments, many fewer neurons were labeled from the PAG than were labeled from the MRF. However, compared to the spinoreticular group, slightly more cells were

TABLE 19

NUMBER OF STT NEURONS LABELED: UPPER CERVICAL SEGMENTS

$\bar{X} \pm$ S.E.M., Number labeled in 15 (25μm) sections from each case

LAMINAE	L-STT (N=11)	M-STT (N=11)
I	9.7 ± 3.5	5.3 ± 2.5
V	52.7 ± 13.5	28.5 ± 3.3
VI	32.7 ± 6.0	11.3 ± 1.5
VII, VIII	44.3 ± 4.1	50.3 ± 6.0
X	0.8 ± 0.4	0.5 ± 0.3
LSN	20.3 ± 5.0	15.1 ± 3.0
LCN	50.0 ± 18.5	1.4 ± 0.7
IBN	47.9 ± 11.6	5.0 ± 1.3
IPSILATERAL		
I	-	2.7 ± 1.9
V	5.9 ± 1.3	10.3 ± 1.5
VI	1.1 ± 0.5	2.5 ± 0.8
VII, VIII	89.2 ± 13.5	68.2 ± 7.2
X	-	-
LSN	3.3 ± 1.0	8.0 ± 1.5
LCN	1.9 ± 1.3	-
IBN	1.6 ± 0.6	1.4 ± 0.7

TABLE 20

NUMBER OF NEURONS LABELED FROM THE PAG OR MRF: UPPER CERVICAL SEGMENTS

$\bar{X} \pm$ S.E.M., Number labeled in 30 (25µm) sections from each case

LAMINAE	SRT (N=12)	SAT (N=9)*
CONTRALATERAL		
I	1.4 ± 0.7	12.7 ± 2.6
V	120.9 ± 21.1	25.1 ± 6.3
VI	20.6 ± 3.8	4.6 ± 1.3
VI, VIII	210.0 ± 35.5	28.5 ± 8.6
X	20.5 ± 5.5	2.9 ± 0.7
LSN	18.6 ± 6.2	23.7 ± 4.8
LCN	1.0 ± 0.5	1.7 ± 1.1
IBN	2.5 ± 1.1	0.3 ± 0.2
IPSILATERAL		
I	3.1 ± 1.0	1.8 ± 0.6
V	129.3 ± 25.8	18.1 ± 4.0
VI	16.8 ± 3.7	1.4 ± 0.7
VII, VIII	205.2 ± 33.0	41.6 ± 13.4
X	14.8 ± 4.8	2.4 ± 0.6
LSN	13.7 ± 2.3	11.1 ± 1.9
LCN	0.7 ± 0.5	1.2 ± 1.0
IBN	0.8 ± 0.4	-

*One animal of this group was not included in this table due to very poor labeling in the upper cervical segments.

labeled in lamina I and equivalent numbers were labeled in the LSN from the PAG. In lamina V, an equally bilateral distribution of labeled cells, spinoreticular and spinoannular, was observed. This was generally true also in laminae VII and VIII. A bilateral labeling of lamina VI neurons was seen from the MRF injections, but few neurons in this region were labeled from the PAG and those few tended to be on the contralateral side. Both types of neurons were also observed, bilaterally, in lamina X. A slight contralateral predominance was seen in the distributions of spinoreticular and spinoannular tract cells in the LSN. Finally, very few neurons were labeled in the internal basilar and lateral cervical nuclei from MRF or PAG injections.

The percentage of STT neurons which were double-labeled in the upper cervical segments is presented in Table 21 for the contralateral side and in Table 22 for the ipsilateral side. Both L-STT and M-STT neurons were consistently double-labeled from the PAG or MRF in the contralateral laminae V,VII and VIII and in the ipsilateral laminae VII and VIII. Double-labeling of STT neurons in the ipsilateral lamina V was variable and generally sparse. The data reported for the lateral ventral horn are subsets of the laminae VII-VIII data. In all four groups, the highest percentages of double-labeled L-STT and M-STT neurons were consistently observed in the lateral ventral horn. These percentages were greater than those of laminae VII-VIII

TABLE 21 PERCENT OF STT NEURONS DOUBLE-LABELED: CONTRALATERAL UPPER CERVICAL SEGMENTS

% (DL/SL&DL STT CELLS, n=NUMBER OF CASES WITH DL CELLS)

LAMINAE	L-STT/MRF (N=6)	L-STT/PAG (N=5)	M-STT/PAG (N=6)	M-STT/PAG (N=5)
I	14% (8/59,n=3)	43% (13/30,n=2)	-	23% (5/22,n=2)
V	19% (61/320,n=5)	16% (39/251,n=5)	36% (61/168,n=6)	15% (18/120,n=4)
VI	26% (46/175,n=5)	9% (8/93,n=3)	18% (13/70,n=5)	-
VII,VIII	30% (81/268,n=6)	30% (65/219,n=5)	36% (103/283,n=6)	16% (44/270,n=5)
X	-	-	-	-
LSN	20% (10/51,n=2)	37% (57/153,n=5)	36% (24/66,n=5)	32% (24/74,n=4)
LCN	-	3% (8/271,n=3)	-	-
IBN	8% (12/149,n=2)	-	-	-
LAT VH	45% (33/74,n=5)	50% (20/40,n=5)	41% (25/61,n=5)	22% (15/69,n=5)

TABLE 22 PERCENT OF STT NEURONS DOUBLE-LABELED: IPSILATERAL UPPER CERVICAL SEGMENTS

% (DL/SL&DL STT CELLS, n=NUMBER OF CASES WITH DL CELLS)

LAMINAE	L-STT/MRF (N=6)	L-STT/PAG (N=5)	M-STT/MRF (N=6)	M-STT/PAG (N=5)
I	-	-	-	-
V	39% (9/23,n=2)	18% (4/22,n=3)	41% (17/41,n=5)	15% (10/65,n=5)
VI	-	-	13% (2/15,n=2)	-
VII,VIII	35% (187/534,n=6)	35% (156/448,n=5)	45% (188/419,n=6)	19% (62/322,n=5)
X	-	-	-	-
LSN	-	30% (5/17,n=2)	22% (7/31,n=3)	-
LCN	-	-	-	-
IBN	-	-	-	-
LAT VH	26% (53/202,n=5)	42% (66/158,n=5)	37% (40/107,n=6)	31% (28/89,n=3)

combined for both the ipsilateral and contralateral sides in the groups with PAG injections. In the groups with MRF injections, the percentages of double-labeled STT neurons seen in the lateral ventral horn exceeded those of laminae VII-VIII on the contralateral, but not the ipsilateral, side. It should be noted, however, that there were more STT neurons, single- and double-labeled, on the ipsilateral side in all four groups and that the ipsilateral versus contralateral trends in the percentage of double-labeled cells were not observed in every case.

There were a few double-labeled STT neurons in lamina I and none in lamina X. In lamina VI, both L-STT and M-STT neurons were double-labeled from the MRF, but only a few were double-labeled from the PAG. Some cases from all four groups exhibited double-labeled STT neurons in the LSN.

Very few double-labeled neurons were observed in the lateral cervical and internal basilar nuclei. In the second cervical segment, the lateral cervical nucleus is gradually replaced by the LSN and the internal basilar nucleus is replaced by lamina VI. It is probable that the few double-labeled cells in these nuclei were located in such areas of transition. Alternatively, the double-labeling could have resulted from uptake of RhS by damaged axons of passage through the injection sites. In any case, the labeling is such a small percentage that it is most likely that neurons labeled from the lateral thalamus in the lateral cervical and internal basilar nuclei project to the thalamus without

issuing axon collaterals to the MRF or PAG.

The pooled data are shown in Figure 37. Presented in this form, the most obvious feature of the labeling observed in the lateral cervical and internal basilar nuclei is the strong predominance of neurons single-labeled from the lateral thalamus. By far the majority of L-STT and M-STT neurons, single- and double-labeled, were located bilaterally, with ipsilateral predominance, in laminae VII and VIII. Up to 40% of these neurons, on the ipsilateral side, were located in the lateral ventral horn region of lamina VIII; on the contralateral side about 30% of the labeled L-STT and M-STT neurons in laminae VII and VIII were located in the lateral ventral horn.

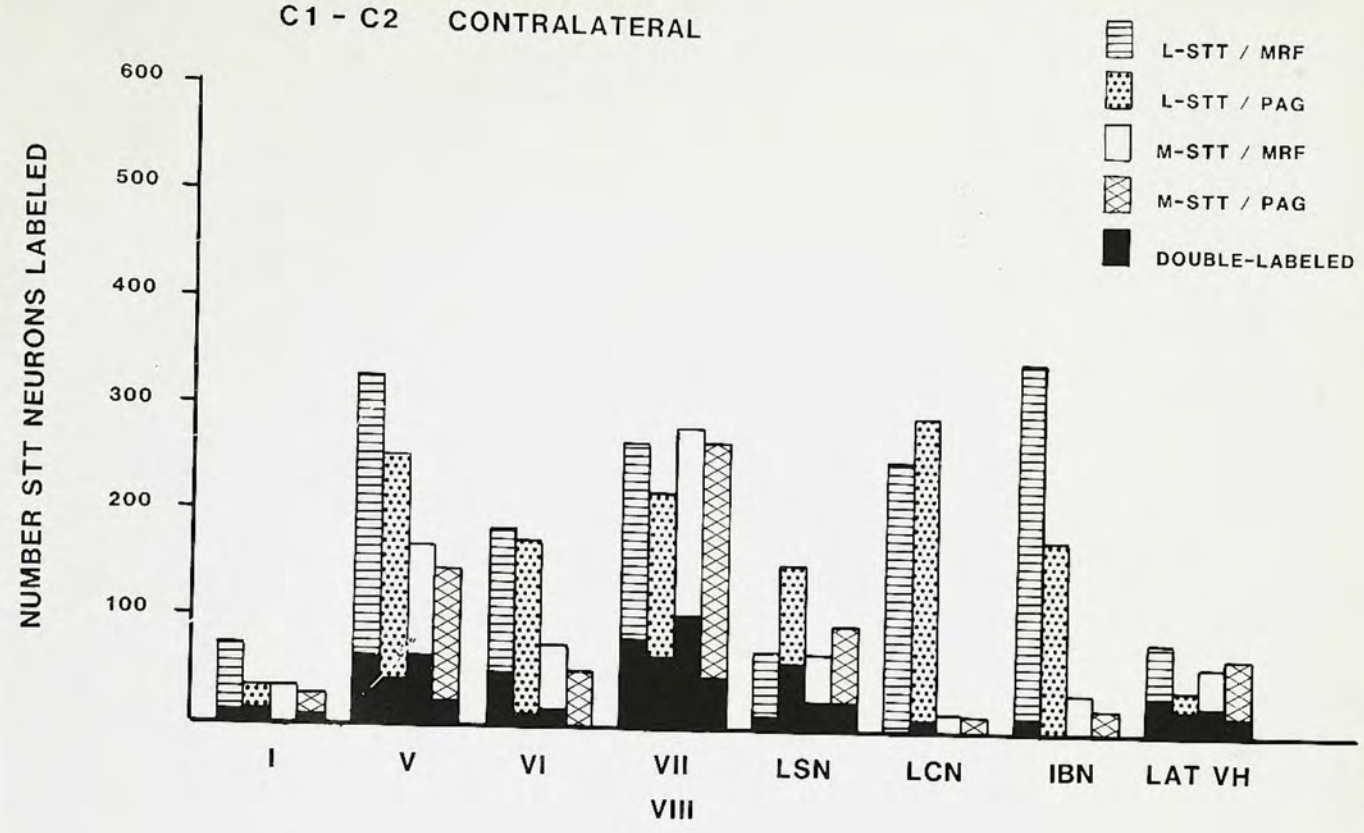
There were more M-STT cells labeled in the LSN, but L-STT neurons were more commonly labeled in laminae V and VI. Except for lamina VI where STT neurons were double-labeled mostly from the MRF, there were no discernible differences between the MRF and PAG groups in terms of double-labeling of STT neurons in the LSN and lamina V.

The illustrations of data from the four individual cases (136,504,129, and 303) are presented in Figures 38-41. In the L-STT/MRF case (136,Fig 38), there was particularly heavy labeling in most of the laminae discussed above. The lateral cervical and internal basilar nucleus contained many single-labeled L-STT neurons. Lamina VI also contained many L-STT cells, some of which were double-labeled. Single- and double-labeled cells were also present

FIGURE 37

Histograms showing the total number of STT neurons in specific spinal laminae which were single- and double-labeled in each experimental group in the upper cervical spinal cord. The data for each group are pooled from: 6 animals in the L-STT/MRF group; 5 in the L-STT/PAG group; 6 in the M-STT/MRF group; and 5 in the M-STT/PAG group. Note that the scale is changed for upper cervical segments due to the heavy cell labeling in these segments.

FIGURE 37


C1 - C2 CONTRALATERAL
L-STT / MRF
L-STT / PAG
M-STT / MRF
M-STT / PAG
DOUBLE-LABELED
NUMBER STT NEURONS LABELED
600
500
400
300
200
100
I
V
VI
VII
VIII
LSN
LCN
IBN
LAT VH

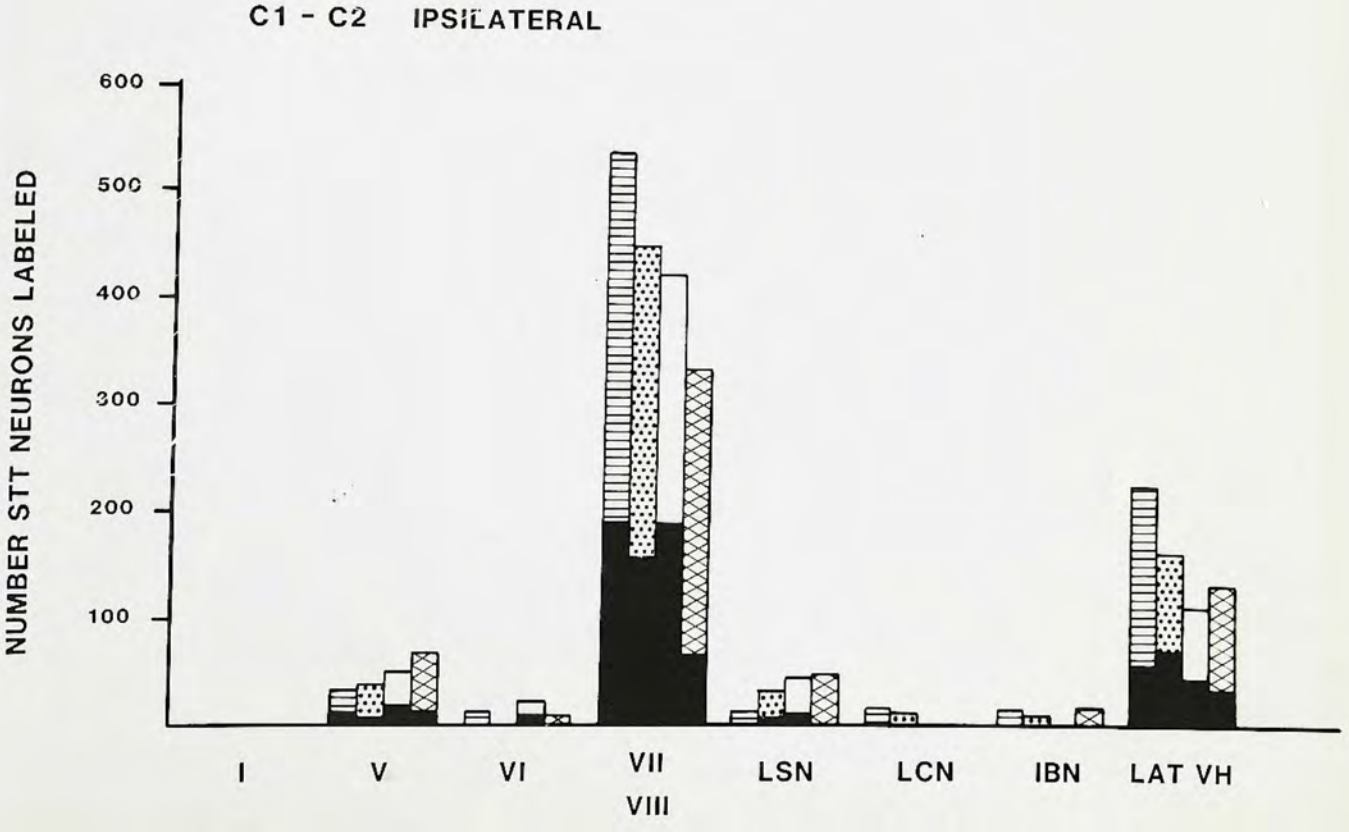
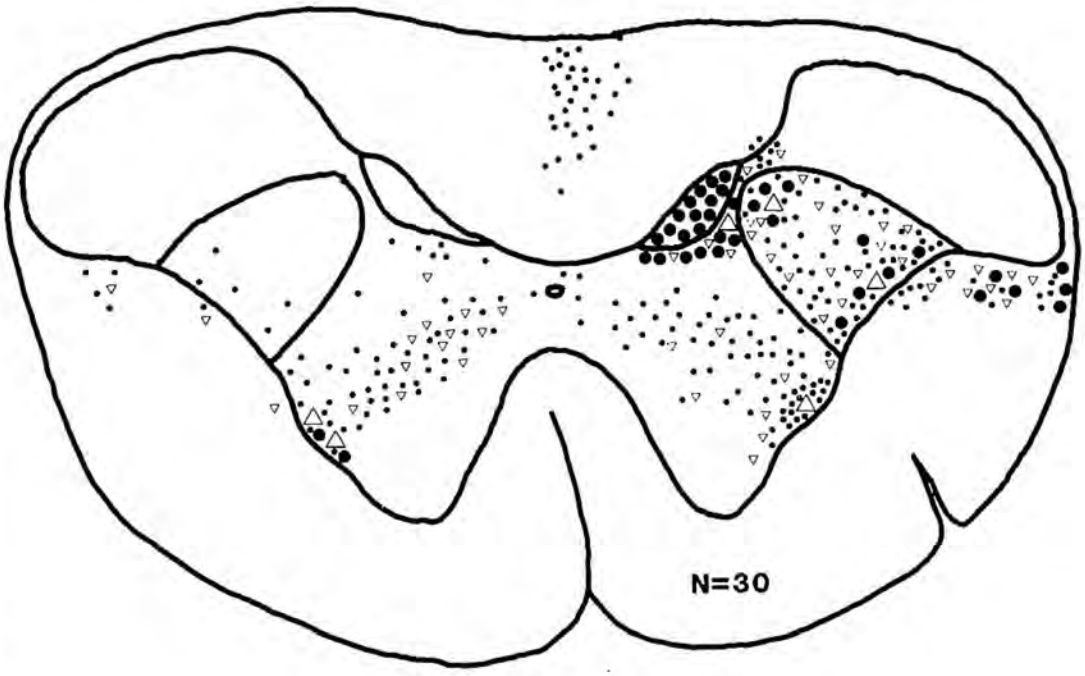

C1 - C2 IPSILATERAL
NUMBER STT NEURONS LABELED
600
500
400
300
200
100
I
V
VI
VII
VIII
LSN
LCN
IBN
LAT VH

FIGURE 38

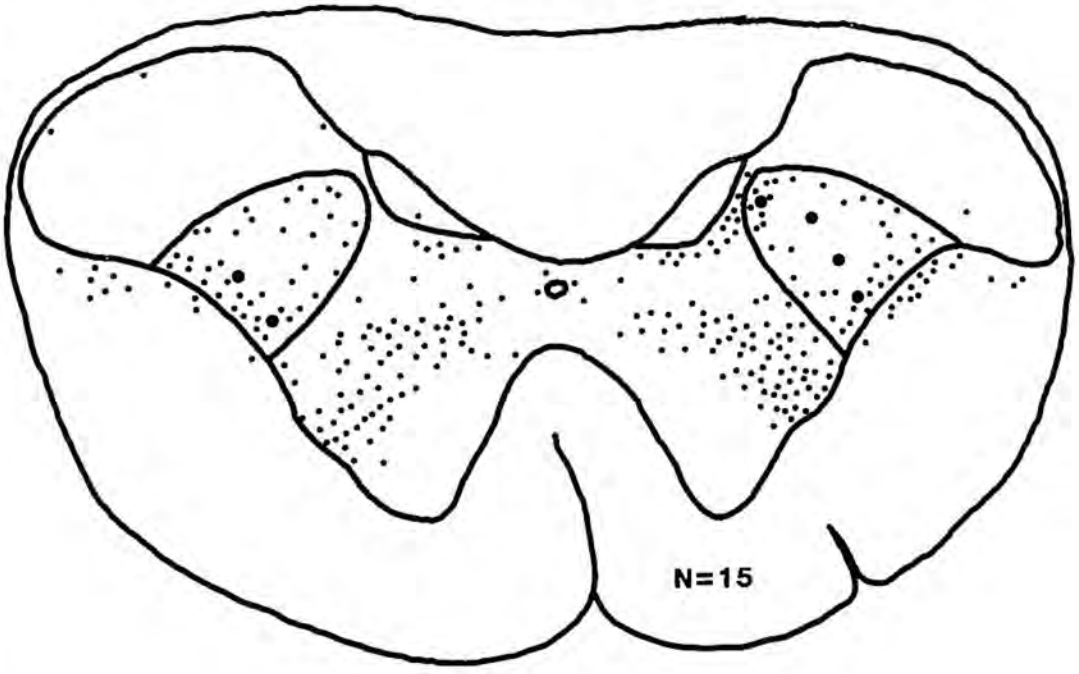
Line drawings illustrating the number and locations of single- and double-labeled neurons in the upper cervical spinal cord of case 136, a L-STT/MRF case. Labeled L-STT neurons are shown in the top drawing and neurons labeled from the MRF are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The large dots and open triangles represent 10 single- or double-labeled neurons respectively. The right side of each drawing is contralateral to the injection sites.

136

L-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SRT CELLS



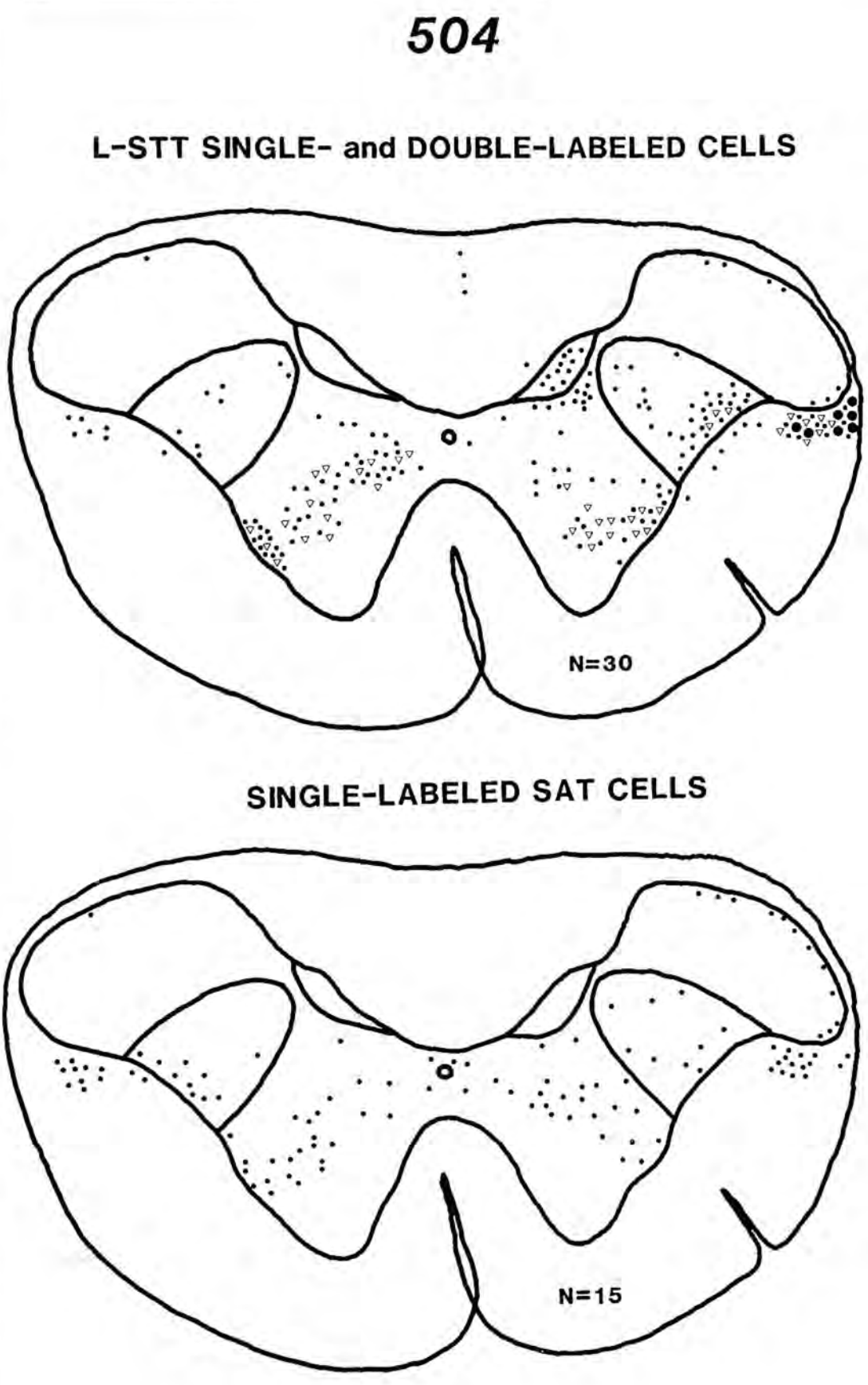
in the most medial part of lamina IV which abuts the internal basilar nucleus. Double-labeled L-STT cells were also seen in the LSN, laminae V,VII and VIII on both sides. Densely packed single- and double-labeled neurons were apparent in the lateral ventral horn on both sides. Finally, single-labeled L-STT neurons were observed in the spinal extension of the nucleus gracilis.

An L-STT/PAG case (504) is shown in Figure 39. Many neurons single-labeled from the lateral thalamus were observed in the lateral cervical nucleus; however, there were less labeled neurons in the internal basilar nucleus than were seen in the previous case. Also, no lamina VI L-STT neurons were double-labeled. In reviewing the data from all the cases, especially the L-STT cases, the heaviest labeling in the internal basilar nucleus is seen in cases in which the thalamic injection site involved the posterior nucleus. It is possible then, based on this observation, that the internal basilar nucleus projects most densely to the posterior nucleus of the thalamus rather than to the VPL. The absence of double-labeling in lamina VI is consistent with the trend that these neurons are most often double-labeled from the MRF, not the PAG.

Lamina V, contralaterally, and laminae VII and VIII, bilaterally, contained L-STT neurons some of which were double-labeled. There were again many such neurons in the lateral ventral horns, especially on the ipsilateral side. Finally, there were a few single-labeled L-STT neurons in

FIGURE 39

Line drawings illustrating the number and locations of single- and double-labeled neurons in the upper cervical spinal cord of case 504, a L-STT/PAG case. Labeled L-STT neurons are shown in the top drawing and neurons labeled from the PAG are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The large dots represent 10 single-labeled neurons each. The right side of each drawing is contralateral to the injection sites.


504
L-STT SINGLE- and DOUBLE-LABELED CELLS
N=30
SINGLE-LABELED SAT CELLS
N=15

lamina I and in the spinal part of nucleus gracilis.

Figure 40 presents the data obtained in the M-STT/MRF case (129). There were no M-STT neurons in the lateral cervical nucleus and very few in the internal basilar nucleus. M-STT neurons, one double-labeled, were observed in lamina VI. Double-labeled neurons were contained in lamina V, contralaterally, and in laminae VII and VIII, bilaterally with ipsilateral predominance. A heavy concentration of single- and double-labeled M-STT neurons were observed in the ipsilateral lateral ventral horn. Finally, there were no double-labeled neurons in the LSN.

The M-STT/PAG case (303,Fig 41) exhibited no labeled M-STT cells in the internal basilar nucleus and only one labeled cell in the lateral cervical nucleus. In addition, only single-labeled M-STT neurons were seen in lamina VI. The majority of double-labeled neurons in this case were located bilaterally in laminae VII and VIII. A higher percentage of M-STT neurons in the contralateral lateral ventral horn were double-labeled than on the ipsilateral side. There was one double-labeled cell each in lamina V and in the LSN.

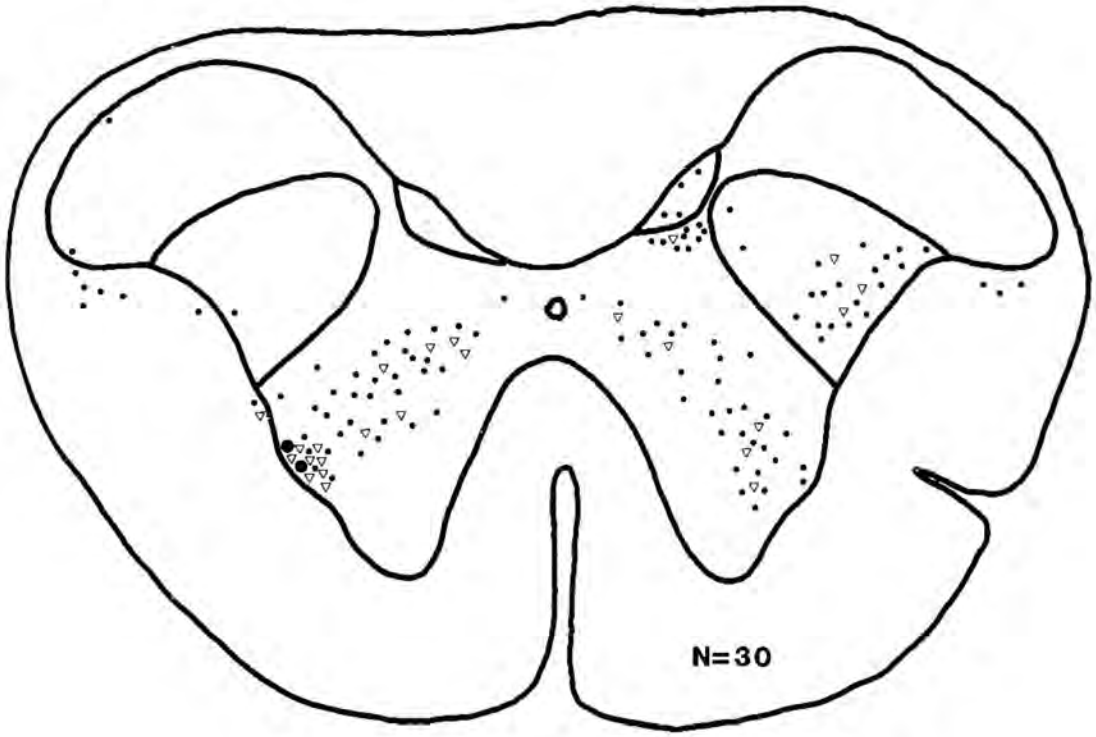
In summary, in the upper cervical segments, there were two regions which seem to project only to the lateral thalamus without axon collaterals to the MRF or PAG. One of these is the lateral cervical nucleus, which is known to project to VPL as part of the spinocervicothalamic tract.

FIGURE 40

Line drawings illustrating the number and locations of single- and double-labeled neurons in the upper cervical spinal cord of case 129, a M-STT/MRF case. Labeled M-STT neurons are shown in the top drawing and neurons labeled from the MRF are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The large dots represent 10 double-labeled neurons each. The right side of each drawing is contralateral to the injection sites.

129

M-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SRT CELLS

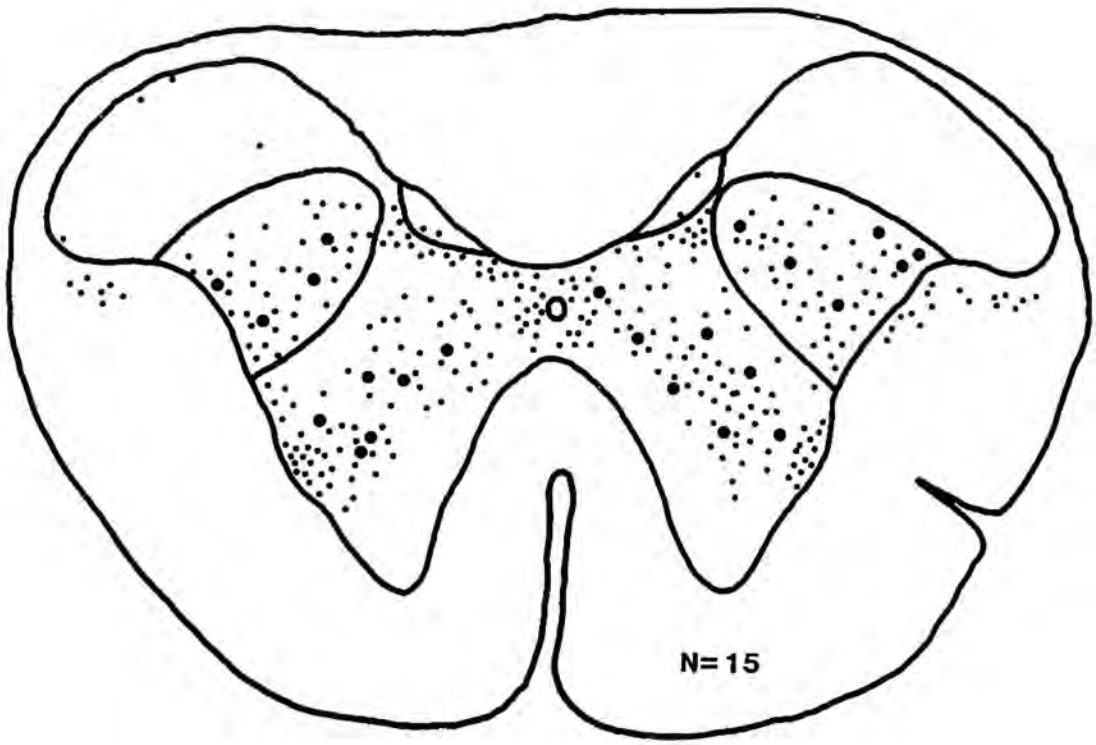
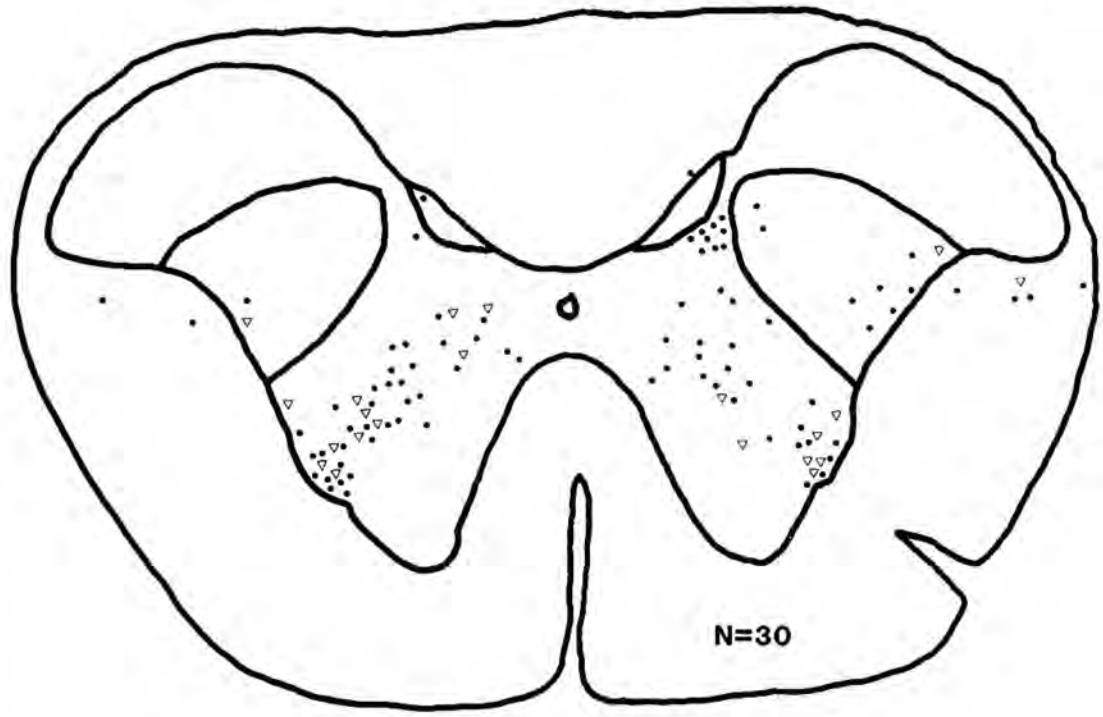


FIGURE 41

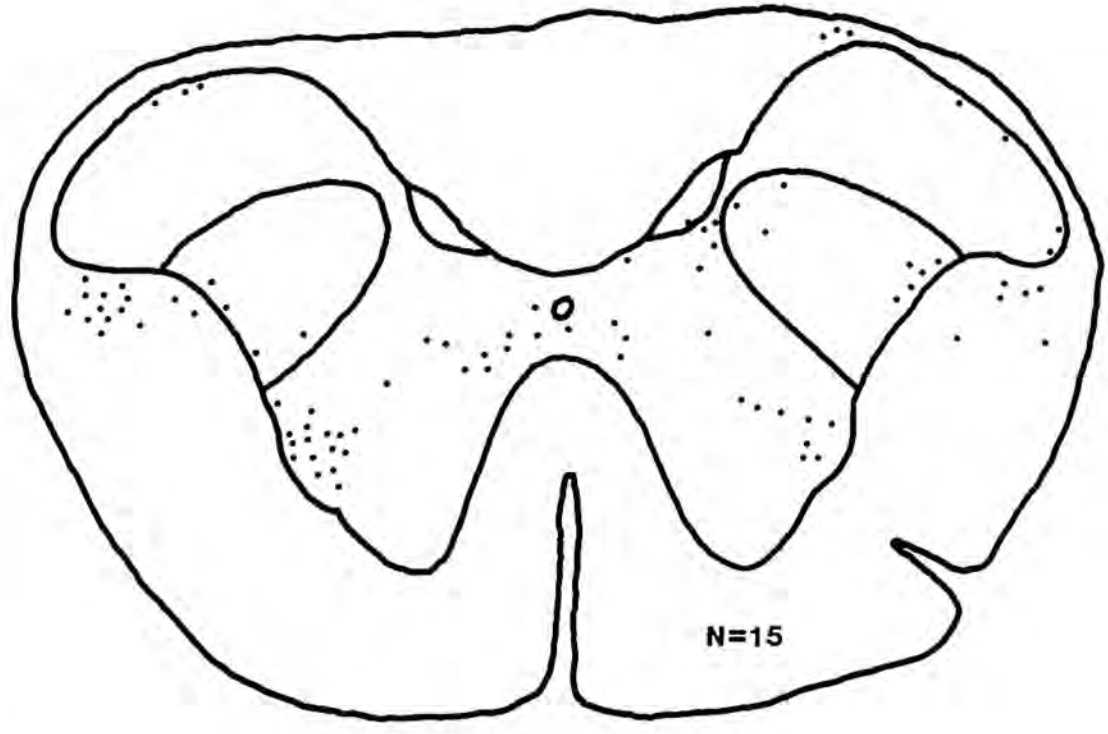
Line drawings illustrating the number and locations of single- and double-labeled neurons in the upper cervical spinal cord of case 303, a M-STT/PAG case. Labeled M-STT neurons are shown in the top drawing and neurons labeled from the PAG are shown in the bottom drawing. The number of sections analyzed to generate these cell plots is shown in the lower right of each drawing. Each small dot represents one single-labeled neuron and each small, open triangle represents one double-labeled neuron. The large dots represent 10 double-labeled neurons each. The right side of each drawing is contralateral to the injection sites.

303

M-STT SINGLE- and DOUBLE-LABELED CELLS



SINGLE-LABELED SAT CELLS



The other is the internal basilar nucleus, which may project mainly to the posterior thalamic nucleus. Surrounding the internal basilar nucleus in lamina VI, there were L-STT and M-STT neurons which tended to be double-labeled from the MRF rather than from the PAG. Many single- and double-labeled M-STT and L-STT cells were located bilaterally, with ipsilateral predominance, in laminae VII and VIII. In all four groups, there was a heavy concentration of STT neurons in the lateral ventral horn, especially on the ipsilateral side, and often a high percentage of these were double-labeled. Single- and double-labeled M-STT and L-STT neurons were also located bilaterally, with contralateral predominance, in laminae V and in the LSN. Finally, a few STT neurons were labeled in lamina I, some of which were double-labeled. Examples of double-labeled neurons in the upper cervical spinal cord are presented in Figure 42. Photomicrographs of labeling in the lateral ventral horn are shown in Figure 43.

TRIPLE-LABEL STUDIES

The purpose of this final experiment was to determine if STT neurons could be triple-labeled from injection of FB, RhS, or DY into the thalamus, PAG, and MRF. The findings are reported here as preliminary observations only because attaining adequate transport of all three tracers proved to be difficult at best. However, a few triple-labeled neurons were seen in three cases. The

FIGURE 42

Photomicrographs (742X) of three double-labeled neurons from the upper cervical spinal cords. The neuron shown in A and B contained FB and RhS respectively and was found in the contralateral ventral horn of case 124 (L-STT/MRF). In C (FB) and D (RhS), the neuron shown was located in lamina V, contralateral to the injection sites , in case 126 (M-STT/MRF). Note how the RhS labeling shows through at the 420 nm wavelength (C) due to the heavy filling of this neuron by RhS. The neuron in E and F (FB and RhS respectively) was located in the ipsilateral ventral horn of case 142 (M-STT/MRF). The FB labeling in E was photographed under 365 nm wavelength light.

FIGURE 42

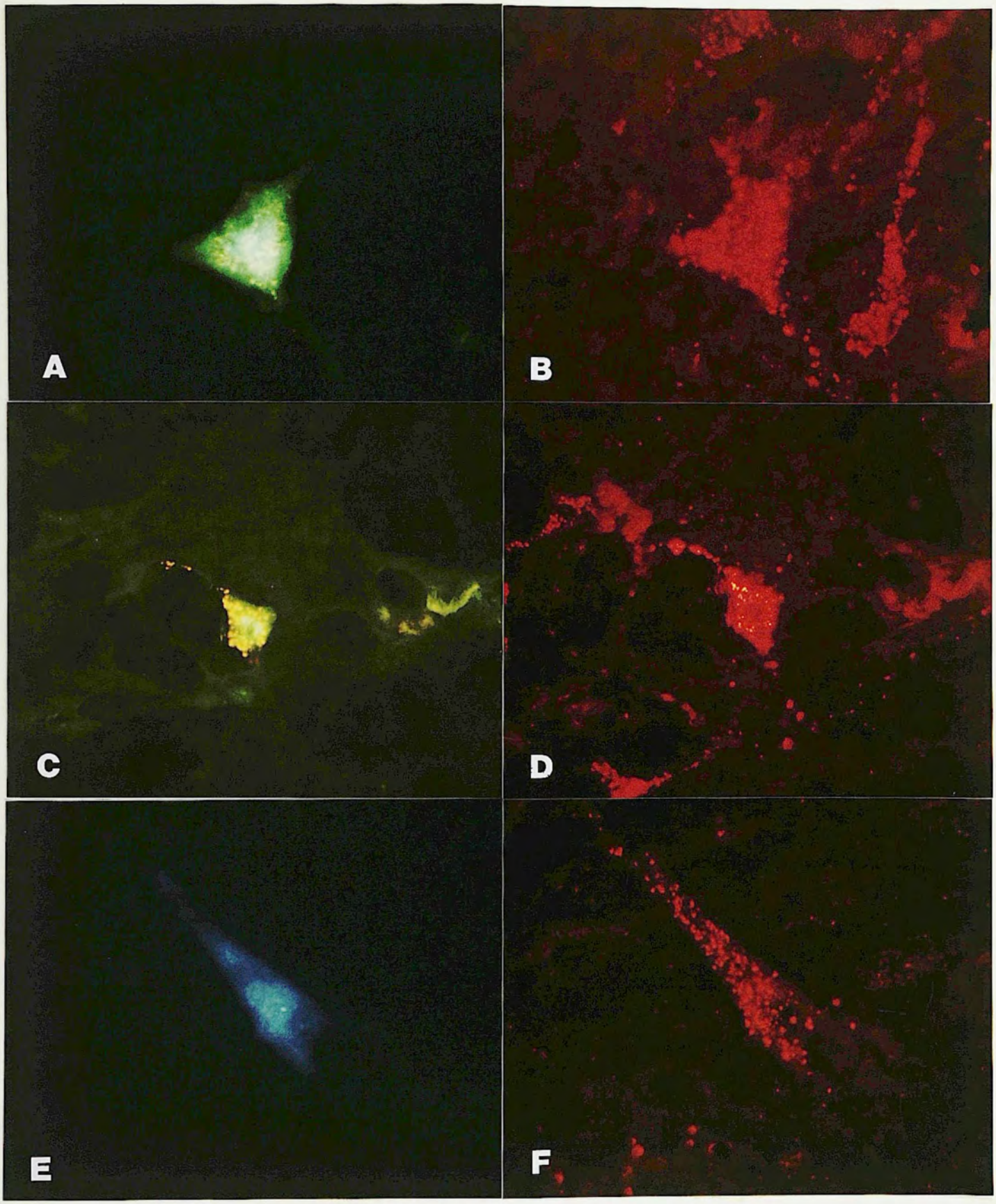


FIGURE 43

Photomicrographs of the same section taken at low magnification (297X) of the ipsilateral lateral ventral horn region in case 502 (L-STT/PAG). In A, taken under 420 nm wavelength light, four FB-labeled neurons are apparent. Two of these neurons are seen to contain a RhS double-label in B (arrows), taken under 550 nm wavelength light.

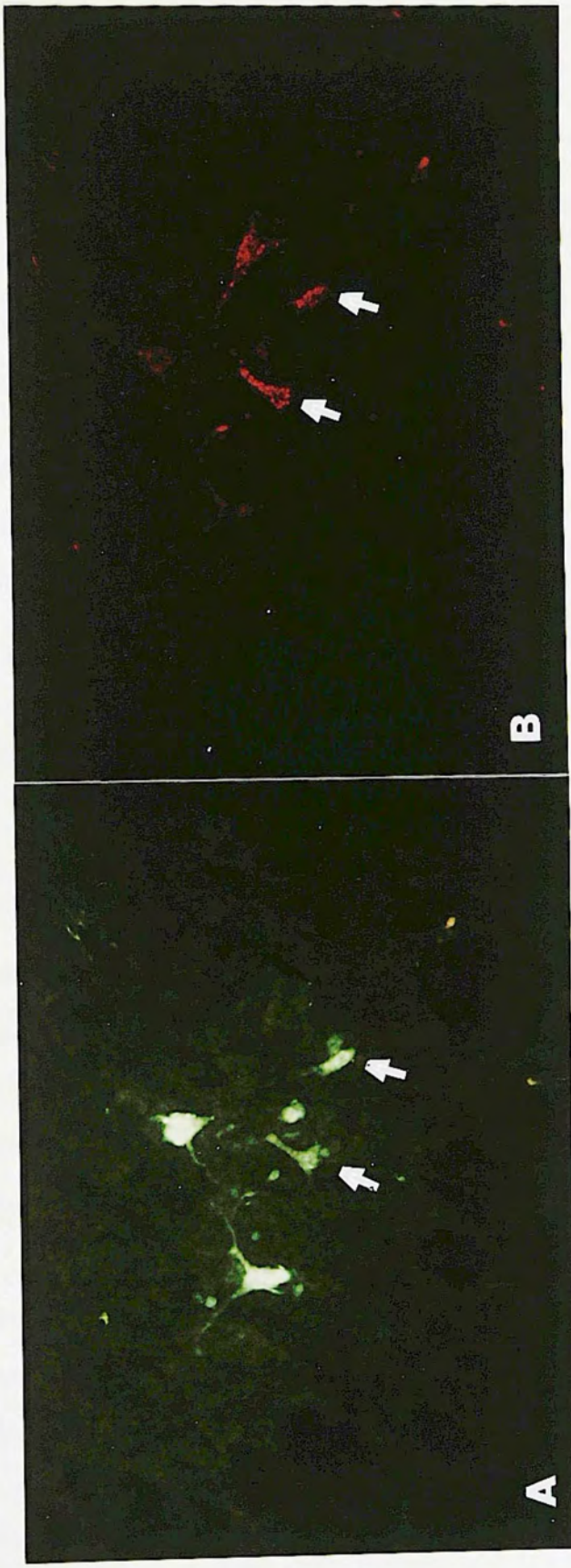


FIGURE 43

data obtained in these animals are described in reference to one illustrated case in which the best labeling was observed.

The injection sites of case 1004 are depicted in Figure 44. The thalamic injection of FB was located in the medial thalamus in a position slightly more ventral than the injection sites of most of the cases in the M-STT groups in the double-label studies. The center of the injection covered the paracentral and centromedian nuclei and impinged upon parts of the central lateral and posterior nuclei. In the PAG, the RhS injection was centered in the ventrolateral part of the PAG at the level of the superior colliculus. At its greatest extent, the injection involved the dorsolateral PAG, its immediately adjacent tegmentum and a small part of the superior colliculus. The DY injection was centered in the nucleus reticularis gigantocellularis at the level of the facial nucleus. Some DY deposit was observed capping the genu of the seventh nerve at the most rostral point of the injection. With the exception of one injection site, which was a more dorsally placed injection into the MRF, the other two cases' injection sites were comparable to those of 1004.

The locations of single-, double-, and triple-labeled STT neurons in the upper cervical segments and cervical enlargement are illustrated in Figure 45. There were two single-labeled STT neurons in the lateral cervical nucleus. Single-labeled cells were also observed in the

FIGURE 44

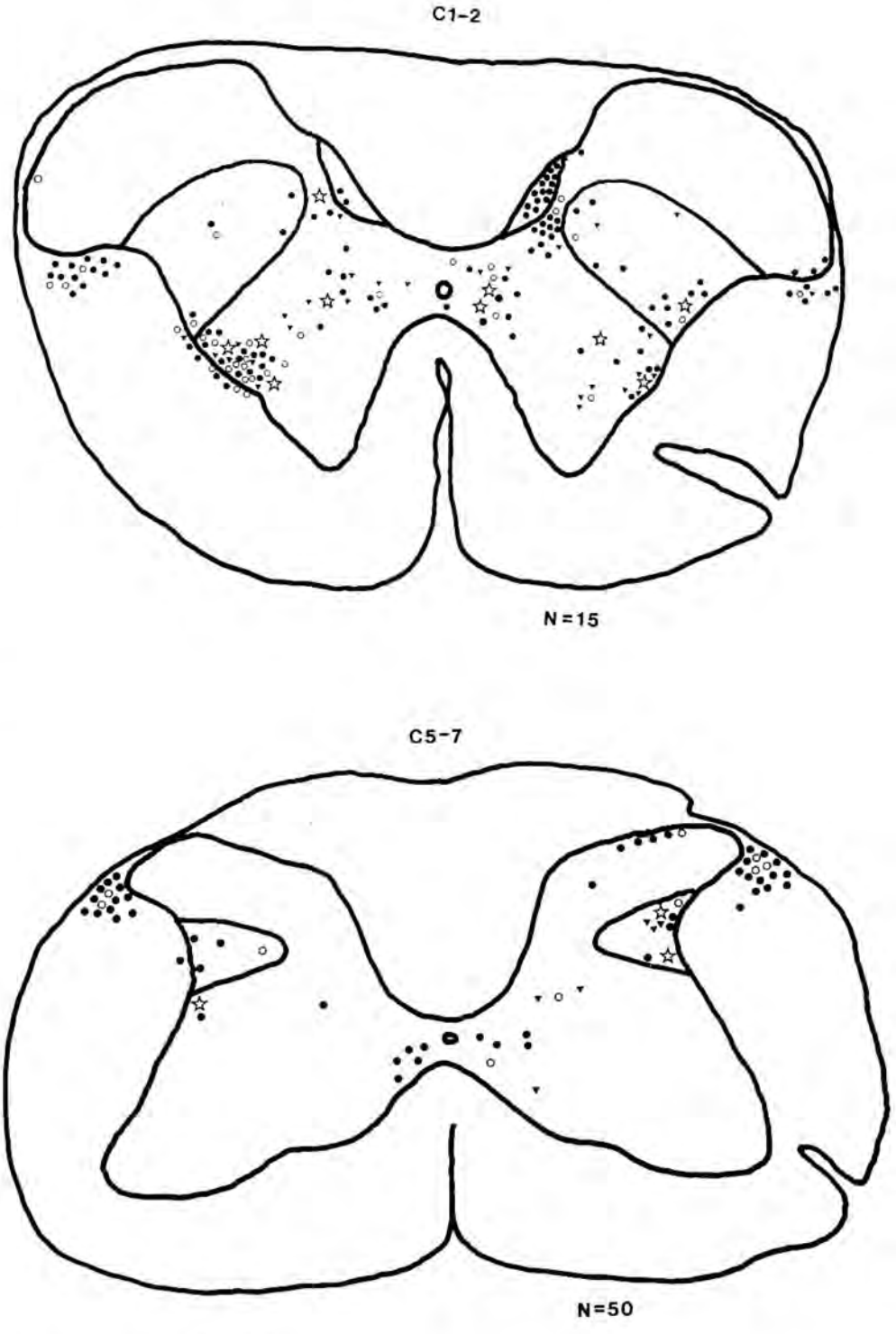
Line drawings illustrating the FB, RhS, and DY injection sites of case 1004. The injection centers are in black and the diffusion zones are represented by the shaded regions. The middle drawings for each site are from the sections containing the largest injection centers. The most rostral extent of each injection center is shown on the right and the most caudal extent is shown on the left.

FIGURE 44

FIGURE 45

Line drawings of the number and locations of single-, double-, and triple-labeled STT neurons in the upper and lower cervical segments of case 1004. The number of sections analyzed to generate these cell plots is shown beneath each drawing. At the bottom left of the figure is a key to the symbols used, each represents one neuron.

1004



internal basilar nucleus, consistent with the thalamic injection involving the posterior nucleus. STT neurons, double-labeled from the PAG or MRF, were seen in the LSN and in laminae V,VII, and VIII on both sides. Triple-labeled STT neurons were observed in laminae V,VII and VIII, contralaterally, and in laminae VI,VII, and VIII, ipsilaterally. In the contralateral lateral ventral horn, there was one triple-labeled STT neuron and a number of STT neurons double-labeled from the MRF. In contrast, on the ipsilateral side of this region, there were three triple-labeled neurons as well as STT neurons double-labeled from the PAG (N=12) and from the MRF (N=5). The other two cases exhibited fewer triple-labeled STT neurons, but all of them were located in lamina VIII.

In cases not illustrated, no triple-labeled STT neurons were found caudal to the upper cervical segments and very few were found in the illustrated case. In all three cases, DY labeling was extremely sparse below the upper cervical level and labeling with FB and RhS was considerably less than that seen in the double-label groups.

Three triple-labeled STT neurons were observed in the cervical enlargement of case 1004. Two of these were located in the contralateral lamina V and one was located ipsilaterally in lamina VI. No such neurons were found in mid-thoracic segments (Fig 46). Only one triple-labeled cell was seen in the lumbar enlargement in lamina X.

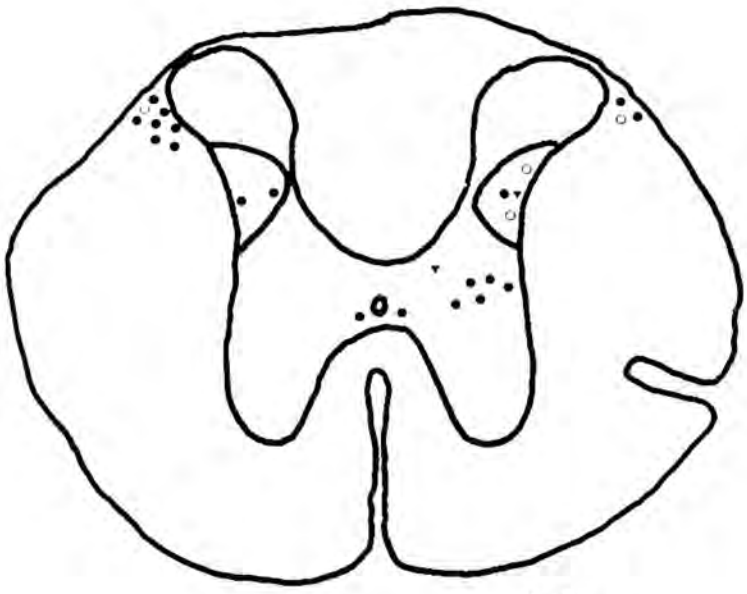
In summary, triple-labeled neurons were present in

FIGURE 46

Line drawings of the number and locations of single-, double-, and triple-labeled STT neurons in the mid-thoracic and lumbar enlargement segments of case 1004. The number of sections analyzed to generate these cell plots is shown beneath each drawing. A key to the symbols used for cell labeling is included in Fig. 45; each represents one cell.

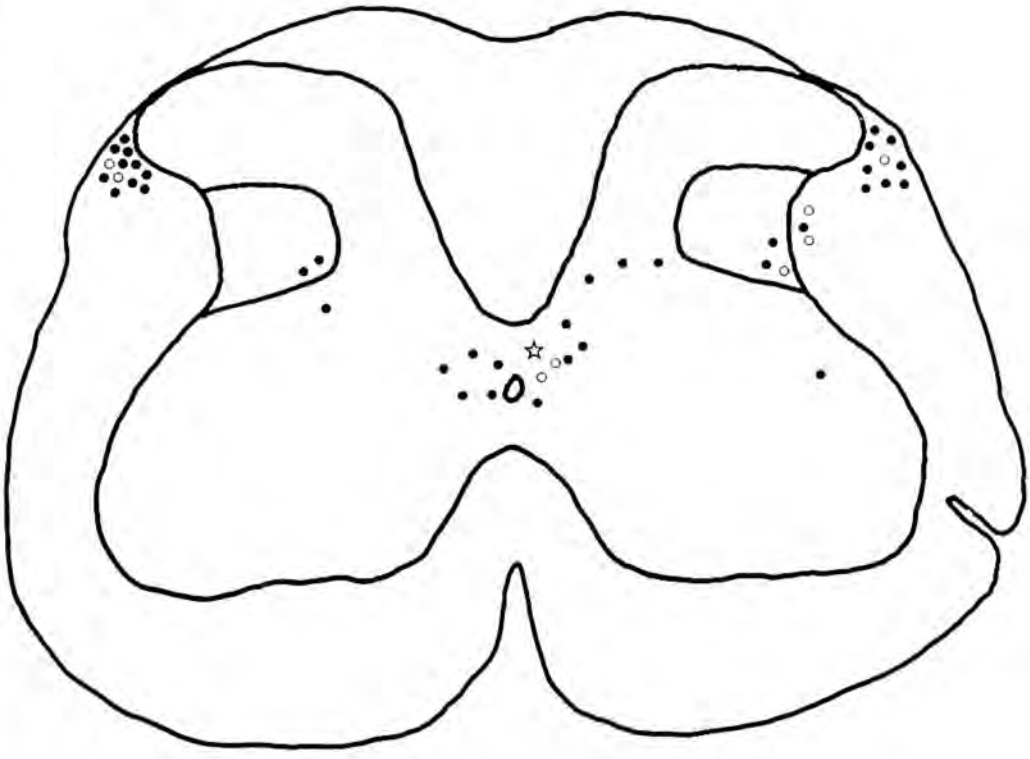
1004

T5-8



N=50

L3-5



N=50

all three cases in the upper cervical spinal cord. Most of these were located bilaterally in lamina VIII. An example of a triple-labeled STT neuron is shown in Figure 47. Finally, only a few such STT neurons were observed in the cervical and lumbar enlargements of one case.

SUMMARY OF MAJOR FINDINGS

At all spinal levels, double-labeled L-STT and M-STT neurons were observed in certain laminae. In those laminae, there was generally little difference in the percentage of L-STT neurons double-labeled versus the percentage of M-STT neurons double-labeled. A few spinal regions were found to contain exclusively single-labeled L-STT but not M-STT cell groups. In the lumbar and cervical enlargements, single- and double-labeled M-STT and L-STT neurons were observed in the contralateral laminae V,VI,VII and VIII and in the LSN. More M-STT than L-STT cells were located bilaterally in these regions. In addition, in the cervical, but not the lumbar, enlargement, STT neurons in the LSN were most frequently double-labeled from the PAG. The contralateral lumbar VMDH contained single-labeled L-STT neurons which may project to the ventral lateral thalamic nucleus. Lamina I contained few labeled STT neurons at lumbar levels. However, many L-STT neurons were found in lamina I in the cervical enlargement. These were double-labeled from the PAG only. There was no cell group in the cervical enlargement which was seen to contain only single-labeled L-

FIGURE 47

Photomicrographs (742X) of a triple-labeled neuron (arrow) found in case 1006. This cell was located in the upper cervical spinal cord in the ipsilateral ventral horn. In A, the neuron is seen to contain FB in the cytoplasm and DY in the nucleus (taken under 365 nm wavelength light). Under 550 nm wavelength light, the RhS triple-label is seen (B). In addition, there is a double-labeled FB-RhS neuron in the field on the right.

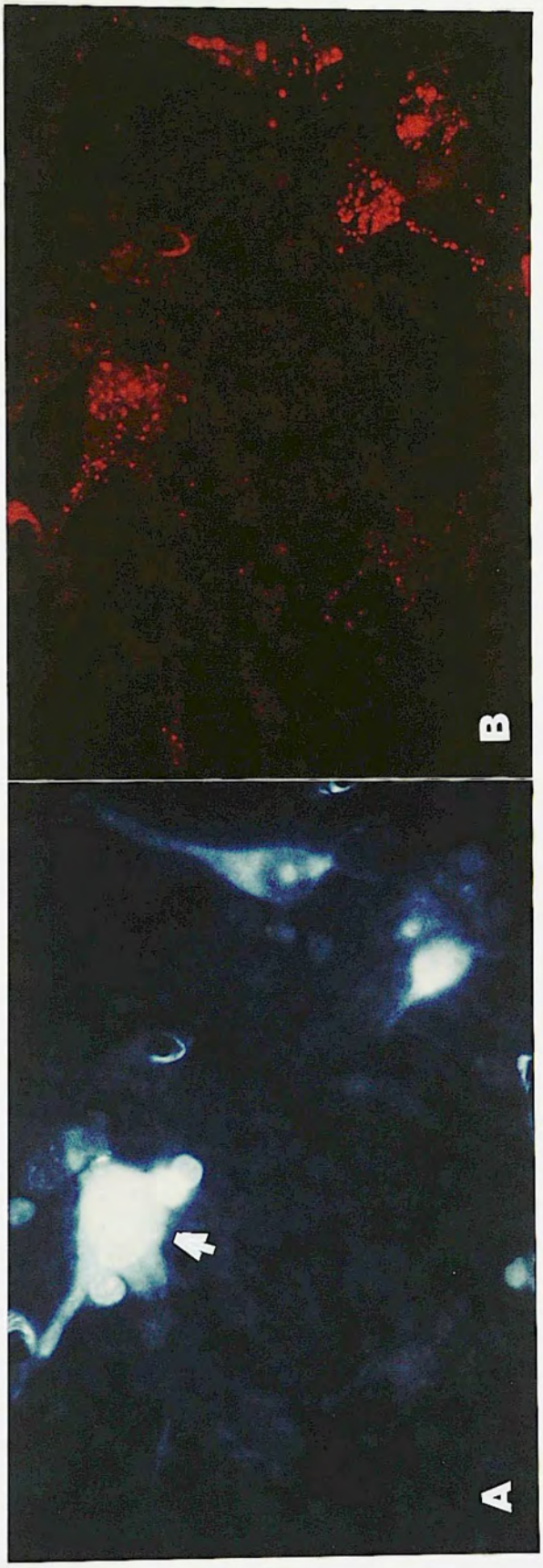


FIGURE 47

STT or M-STT neurons.

The fewest number of labeled STT neurons were found in mid-thoracic segments. Both L-STT and M-STT cells, some of which were double-labeled from the MRF and PAG, were found in laminae V,VII and VIII. As in the cervical enlargement, STT neurons in the LSN at mid-thoracic levels were most commonly double-labeled from the PAG.

The greatest number of labeled STT neurons were observed in the upper cervical spinal cord and the pattern of this labeling differed in a number of ways from that seen in other spinal levels. M-STT and L-STT neurons, single- and double-labeled, were present bilaterally, with ipsilateral predominance, in laminae VII and VIII. Many of these neurons were densely packed into the lateral ventral horn portion of lamina VIII. In general, the highest percentages of STT neurons which were double-labeled were seen in the upper cervical laminae VII and VIII. Single- and double-labeled STT neurons in lamina V and in the LSN were located mainly on the contralateral side. Few STT cells were labeled in lamina I.

Almost all the neurons labeled in the contralateral lateral cervical nucleus were single-labeled from lateral thalamic injections. The internal basilar nucleus almost entirely contained single-labeled L-STT neurons and the heaviest labeling was associated with involvement of the posterior thalamic nucleus in the injection sites. Surrounding the lateral boundary of the internal basilar

nucleus was a region consisting of lamina VI and a small, medial, part of lamina IV in which both L-STT and M-STT neurons, some double-labeled from especially the MRF, were found.

Finally, in the triple-label study, few neurons were found which contained all three tracers. The majority of such cells were located in the upper cervical spinal cord, bilaterally, in lamina VIII. There was, however, a general paucity of labeling in the triple-label cases which was particularly severe for labeling with DY.

DISCUSSION

The data presented here demonstrate that the STT in the rat is a complex system in which both L-STT and M-STT neurons contribute axon collaterals to other brainstem regions. In order to infer the role of this multi-component STT in the processing of somatosensory information, it is necessary to consider these data not only in the context of previous anatomical studies, but also in terms of current knowledge regarding the physiological response properties of specific parts of this system. Such a consideration, however, must also deal with the spinoreticular and spinomesencephalic/spinoannular tracts.

SPINORETICULAR TRACT

The present study defined the locations of spinal neurons which were labeled from tracer injections into the MRF in a total of 12 animals. Such spinoreticular neurons were mainly located bilaterally, with contralateral predominance, in laminae V, VII and VIII in the spinal enlargements and mid-thoracic segments. A bilateral distribution in these laminae was observed in the upper cervical spinal cord. In addition, at all spinal levels examined, spinoreticular neurons were found in the LSN and in laminae VI and X. Many previous retrograde tracing studies in cat, monkey and rat are in accord with the present findings in laminae V, VII, VIII and X (Gallager and Pert,1978; Abols and Basbaum,1981; Andrezik et al.,1981;

Kevetter et al.,1982; Chaouch et al.,1983; Peschanski and Besson,1984; Pechura and Liu,1986). Laminae containing labeled neurons were dissimilar only in those studies in which the MRF injection sites differed markedly from those in the present study. For example, HRP injections into the lateral reticular nucleus resulted in labeled neurons in laminae I, III, IV, and the medial, non-reticulated, part of lamina V (Chaouch et al.,1983; Menetrey et al.,1983). In addition, HRP injections into the nucleus paragigantocellularis lateralis, ventral to the present sites, labeled a number of cells in lamina IV (Andrezik et al.,1981).

Labeled neurons in the LSN have been reported in a few studies in rat following tracer injections into the MRF (Chaouch et al.,1983; Pechura and Liu,1986). This discrepancy may reflect differences in the transport properties of the tracer substances. With one exception (Pechura and Liu,1986), HRP has been the tracer used in all the previous work on the spinoreticular tract. A possible advantage of fluorescent tracers is that they do not depend upon histochemical reactions to be visualized, whereas visualization of the HRP reaction product is dependent upon a number of variables, including the amount of tracer in the cell body. Also there is some direct evidence that HRP, in certain brain regions, labels fewer neurons than true blue, a fluorescent tracer similar to fast blue (Sawchenko and Swanson,1981; Cavada et al.,1984). Differences in tracer

properties may be of particular importance in labeling of neurons, such as those in the LSN, which have small cell bodies and thin, unmyelinated, axons (Gwyn and Waldron,1968,1969; Menetrey et al.,1980). These cells may transport less of any tracer than larger neurons. With these considerations in mind, it is probable that the discrepancy in LSN labeling is not indicative of any major conflict between the present studies and those of previous investigators.

The physiological response properties of lumbar spinal neurons antidromically activated from the MRF have been investigated in cat and rat. In the cat, some spinoreticular neurons are of the wide-dynamic-range type which respond to light mechanical stimulation of the skin, but respond maximally to noxious stimulation such as pinch (Fields et al.,1975,1977; Maunz et al.,1978). Fields and his coworkers (1977) observed the majority of such neurons to be located in the dorsal horn, including lamina V. Other spinoreticular neurons responded to pressure to deep structures, such as muscles and joints, and were located in the dorsal horn and in laminae VII and VIII (Fields et al.,1975,1977). A third general type of neuron was found exclusively in laminae VII and VIII. These neurons had complex receptive fields and were often inhibited by input from cutaneous and deep structures (Fields et al.,1977; Maunz et al.,1978).

The response properties of spinoreticular neurons in

the rat have been studied by Menetrey and his colleagues using antidromic stimulation sites throughout the reticular formation (1980) and in the lateral reticular nucleus (1984a). In their first study, these workers found a more heterogeneous population of neurons than was seen by Fields and his colleagues (1977). Approximately 40% of these cells were wide-dynamic-range neurons, however, 20% were high-threshold type cells which respond exclusively to intense, mostly noxious, stimuli. The remaining neurons either responded only to light touch (26%) or, as observed in the cat, exhibited complex properties which included inhibition by cutaneous and deep stimulation. Neurons in the LSN differed from those in the dorsal horn in that they had slower conduction velocities, were driven only by stimulation of subcutaneous or deep structures, and often projected bilaterally to the reticular formation. Spinal neurons antidromically activated from the lateral reticular nucleus were characterized in the second study of Menetrey and his colleagues (1984a). These cells were located in laminae I, III, IV, the medial part of lamina V, and in layers VII and VIII. Two thirds of the neurons in lamina V were reported to be of the wide-dynamic-range variety. However, unlike the findings of Fields et al., almost half of the neurons in laminae VII and VIII responded only to noxious stimuli and 25% could not be driven by any type of peripheral stimulation. The remaining cells were either responsive to deep stimulation of joints or inhibited by

peripheral stimulation.

It is unfortunate that so few studies have used electrophysiological techniques to study spinoreticular neurons. The most serious difficulties are presented in the work of Menetrey et al. (1980,1984a). Their antidromic stimulation sites in the reticular formation were almost entirely in the pons and midbrain (only 7 of 86 sites were located in the n. reticularis gigantocellularis; see Fig 1 from their 1980 paper) and, thus they do not correspond to the present injection sites nor to most of those from previous retrograde tracing studies. Some of their stimulation sites, besides involving spinomesencephalic tract axons, would very likely involve passing STT axons (Mehler,1969; Bjorkeland and Boivie,1984). Their activation sites in the lateral reticular nucleus (Menetrey et al.,1984a) are also compromised by nearby STT axons of passage. In fact, the authors state that half of the spinal neurons which they activated from the thalamus as a control were also activated from their stimulation in the lateral reticular nucleus.

In summary, both anatomical and physiological evidence generally support the present findings of spinoreticular neurons in laminae V, VII, VIII and in the LSN. These neurons almost certainly play a role in sensory processing since electrophysiological studies have found that some of them respond to a variety of cutaneous and deep stimulation, including light touch and nociceptive

stimulation.

SPINOMESENCEPHALIC / SPINOANNULAR TRACT

Spinal neurons labeled from tracer injections into the PAG (N=10) were observed in the present study in lamina I, lateral V, VI-VIII, X, and in the LSN at all spinal levels examined. The majority of these cells were located contralateral to the injections. In the upper cervical segments, however, there was an equal, bilateral distribution in lamina V and an ipsilateral predominance in laminae VII and VIII. Thus, with the exception of dense lamina I labeling, these labeled neurons were found in a distribution similar to that observed following MRF injections. These data are in good agreement with previous retrograde tracing studies of the spinoannular and spinomesencephalic tracts in monkey (Willis et al.,1979), cat (Wiberg and Blomqvist,1984; Hylden et al.,1986a), and rat (Menetrey et al.,1982; Liu,1983,1986; Pechura and Liu,1986) except for certain observations in the upper cervical segments.

Some previous studies have reported dense labeling of neurons in the lateral cervical nucleus (Hylden et al.,1986a), internal basilar nucleus and spinal parts of the dorsal column nuclei following HRP injections into midbrain regions sometimes including the PAG (Menetrey et al.,1982; Wiberg and Blomqvist,1984). Dense labeling in these regions was not observed in the present studies; instead,

substantial numbers of labeled neurons in these areas were only observed if the PAG injections were large and extended into the midbrain tegmentum. The cases in which this occurred were not included in the present results due to the possibility of labeling STT axons of passage. Nevertheless, the discrepancy of labeling in these regions might well reflect differences in the location of the injection sites. In the cat, projections of lateral cervical and dorsal column nuclei to the intercollicular nucleus and to the deep layers of the caudal superior colliculus have been shown in retrograde and anterograde studies (Berkley et al.,1980; Flink et al.,1983). A projection from the rostral dorsal column nuclei to the collicular plate, but not to the PAG, was also reported in the degeneration studies of Lund and Webster (1967a) in the rat. These data, however, were not illustrated and seemed to constitute only an incidental finding.

It is notable that in the previous studies showing dense labeling in these areas, the tracer injections were located in the caudal superior colliculus (Berkley et al.,1980; Wiberg and Blomqvist,1984), at inferior collicular levels including only part of the PAG (Menetrey et al.,1982), or in the parabrachial area beneath the inferior colliculus (Hylden et al.,1986a). These sites are considerably more caudal than those in the present and other studies in which only a few labeled neurons were observed in these nuclei (Beitz,1982; Pechura and Liu,1986). It is,

therefore, possible that these areas do not project to the PAG but, rather, project to the caudal tectum and intercollicular nucleus of rats and cats.

In a previous double-labeling study, a few neurons in the dorsal column nuclei and 40% of labeled neurons in the lateral cervical nucleus were double-labeled following tracer injections into the thalamus and tectum of cats (Berkley et al.,1980). However, the question of whether these projections in rat are from STT axon collaterals remains to be resolved.

Knowledge of the physiological response properties of spinomesencephalic neurons is extremely sparse. A recent study characterized the response properties of 90 neurons in the lumbar enlargement of cats which were antidromically activated from the midbrain including numerous sites in the PAG (Yezierski and Schwartz,1986). The responses of these cells were contained in four general categories. Those exhibiting wide-dynamic-range characteristics accounted for 42% of the sample. Twenty five percent responded only to nociceptive stimuli and 23% had low spontaneous activity and were unresponsive to peripheral stimulation. The remaining neurons responded to deep stimulation of muscles and joints. The data correspond in many respects to those from studies of spinoreticular tract neurons (Fields et al.,1975,1977; Maunz et al.,1978; Menetrey et al.,1980). Both tracts seem to contain a substantial number of wide-dynamic-range type neurons. They also contain some neurons

which are nociceptive-specific, some which respond to deep stimulation, or some which are unresponsive to any peripheral stimulation. In addition, there is close agreement between these studies in the average conduction velocities observed (about 45 m/s) indicating that many of the neurons of origin for both tracts are similar in size.

In sharp contrast to these general characteristics, lamina I neurons antidromically activated from the midbrain have been found to be almost entirely nociceptive-specific (Hylden et al.,1986b). Sixteen of these neurons were also tested with stimulation sites in the thalamus and half of these could be driven from both the thalamus and the midbrain with antidromic response characteristics consistent with the view that these were collateral projections. The neurons in this study had an average conduction velocity of 6.9 m/s. The intracellular filling of some of these physiologically identified cells with HRP confirmed that they had thinly myelinated axons and varied cellular morphology. Only a few of these neurons exhibited wide-dynamic-range characteristics.

In summary, the locations of neurons labeled from the PAG in the present study are consistent in the spinal enlargements and mid-thoracic segments with the findings of previous retrograde tracing studies. These findings confirm that laminae V-VIII, X, and the LSN contain both spinoreticular and spinoannular tract neurons. Some neurons in these laminae have been shown to project to both the MRF

and midbrain sites such as the PAG via axon collaterals (Fields et al.,1977; Pechura and Liu,1986). There is evidence that the lateral cervical nucleus, the internal basilar nucleus, and the spinal extensions of the dorsal column nuclei project to the caudal tectum, but not to the PAG. Such projections to the tectum raise interesting questions for future research since they may comprise an additional collateral pathway of STT neurons. Finally, recent physiological studies have demonstrated many similarities in the response properties of spinomesencephalic and spinoreticular neurons. These characteristics strongly support the idea that both of these tracts function in the processing of somatosensory information. However, the differences between the two tracts, most clearly seen in the lamina I projection to the midbrain, suggest that certain functional roles may be separate and non-overlapping.

SPINOTHALAMIC TRACT

The present study is the first to examine medially versus laterally projecting STT neurons in terms of their collateral projections to other brainstem regions. The specific patterns of single- and double-labeled STT neurons defined by this investigation strongly suggest that the STT system includes direct and branched projections. The segmental distributions of STT neurons varied; some cells were observed in similar positions throughout the

rostrocaudal extent of the spinal cord, while others were found in discrete groups, specific to particular spinal segments. It is useful, therefore, to consider the labeling patterns found in specific spinal laminae and nuclei.

Lamina I

Very few L-STT or M-STT neurons were found in this lamina except in the cervical enlargement where the majority were labeled from lateral thalamic injections. Consistent with the labeling of many spinoannular neurons in layer I, almost a third of the L-STT and M-STT neurons in this lamina in the cervical enlargement were double-labeled from the PAG. None were double-labeled from the MRF. These data are in agreement with other single- and double-label investigations in the rat (Giesler et al.,1979a; Kevetter and Willis,1983; Granum,1986; Liu,1986). However, Kemplay and Webster (1986) reported only a few labeled neurons in lamina I of the cervical enlargement following large HRP injections into the thalamus. Possible explanations for this discrepancy are not obvious. Also unlike the present data, some workers have reported many HRP-labeled neurons in upper cervical lamina I, part of which is contained in the spinal trigeminal nucleus caudalis (Kevetter and Willis,1983; Granum,1986). Since in both these studies the thalamic injection sites were large, the labeling could have resulted from involvement of the ventral posteromedial nucleus in the injection sites.

Recently, it has been found that many layer I neurons, especially in the cervical enlargement, can be retrogradely labeled following tracer injections into the hypothalamus of rats (Burstein et al., in press). In addition, electrophysiological study of these neurons suggest that they may issue an axon branch to the thalamus. It is possible, then, that some of the lamina I neurons labeled in the present study are part of this spinohypothalamic tract. However, the STT labeling distribution of the present report differs in some important ways from the pattern observed by Burstein and his colleagues. For example, many lamina X neurons were labeled from the hypothalamus and an almost equal bilateral distribution for the labeling in lamina I, V, and the LSN was observed in the spinohypothalamic tract study. In contrast, very few lamina X neurons were labeled in the present study and, except for the LSN, there was a contralateral predominance to labeled STT neurons in lamina I and V. Nevertheless, some of the labeled neurons observed in lamina I may belong to the spinohypothalamic tract. It would be interesting to know, based on the large numbers of spinoannular tract neurons and double-labeled STT/PAG neurons found in lamina I, whether spinohypothalamic neurons issue axon branches to the PAG.

The presence of STT neurons in lamina I differs in cat and monkey compared to the rat. Unlike the present results, tracer injections into the thalamus of cat and

monkey resulted in the labeling of many lamina I neurons in the lumbar enlargement (Carstens and Trevino,1978a; Willis et al.,1979; Hayes and Rustioni,1980). However, similar to the present findings in the cervical enlargement, it was found that L-STT neurons in lamina I outnumber M-STT cells (Carstens and Trevino,1978a; Willis et al.,1979). It is important to note that, among studies which discriminated between medially versus laterally projecting STT neurons, there is agreement among species that a larger number of L-STT neurons are located in the dorsal horn, whereas M-STT neurons are most common in the ventral horn and intermediate zone (Carstens and Trevino,1978a; Willis et al.,1979; Giesler et al.,1979a; present study). In cat and monkey, unlike in the rat, this general trend in the lumbar enlargement is attributable to the heavy labeling of L-STT neurons in lamina I.

Laminae II - IV

In the present study, only a few STT neurons were found in laminae II-IV and none of these were double-labeled. These results are in accord with most previous studies of the STT in cat (Carstens and Trevino,1978a), rat (Giesler et al.,1979a; Kevetter and Willis,1983; Kemplay and Webster,1986; Liu,1986), and monkey (Willis et al.,1978,1979; Hayes and Rustioni,1980). However, Granum (1986) observed a number of STT neurons in upper cervical lamina III (dorsal portion) and IV (ventral portion). Since

this unusual labeling coincided with atypically heavy labeling in upper cervical lamina I, it is possible that both regions were labeled from the ventral posteromedial nucleus.

Lamina V

In all segments examined, there were many STT neurons labeled in lamina V, mostly on the contralateral side. In this region of the lumbar enlargement and upper cervical segments, L-STT neurons outnumbered M-STT neurons. Both L-STT and M-STT cells in this layer were found to be double-labeled from the MRF and from the PAG. In addition, triple-labeled STT neurons were observed in lamina V at upper and lower cervical levels. The highest percentages of double-labeled STT neurons were seen in the cervical enlargement (20-30%), while the other segments exhibited slightly lower percentages (10-25%). These data correspond to previous findings in single-label (Giesler et al.,1979a; Granum,1986; Kemplay and Webster,1986) and double-label studies (Kevetter and Willis,1983; Liu,1986) of the STT in rat. These findings are also similar to single-label data reported in monkey (Willis et al.,1979; Hayes and Rustioni,1980). In cat, only large thalamic injections or injections into the posterior nucleus of the thalamus resulted in a few labeled STT neurons in lumbar lamina V (Carstens and Trevino,1979a). In contrast, few lamina V neurons were labeled in this study following discrete medial

or lateral thalamic injections. It would seem, then, that the labeling observed in lamina V in the rat more closely resembles that observed in the monkey rather than that in the cat.

The present results identify lamina V as a region which contains overlapping populations of L-STT, M-STT, spinoreticular, and spinoannular tract neurons. Some of these neurons belong to two or more of these tracts as evidenced from the presence of double- and triple-labeled STT neurons in this layer. Spinohypothalamic neurons have also been found in abundance in lamina V and physiological evidence indicates that some of these may also belong to the STT (Burstein et al.,in press).

Laminae VI-VIII: Enlargements and Mid-Thoracic Segments

In the mid-thoracic segments, labeled STT neurons were located mainly in the contralateral laminae VII and VIII. In the lumbar and cervical enlargements, labeled STT neurons were common in laminae VI-VIII, mostly on the contralateral side. Unlike the findings of Giesler et al.(1979a), there were about equal numbers of L-STT and M-STT neurons in these layers in the lumbar enlargement. However, the data from the cervical enlargement agree with these previous authors in that M-STT cells were more numerous than L-STT neurons. As observed in previous studies (Giesler et al.,1979a; Kevetter and Willis,1983; Granum,1986; Kemplay and Webster,1986; Liu,1986), the

smallest numbers of STT neurons were generally in mid-thoracic segments and the greatest numbers of labeled neurons were in lumbar segments in laminae VI-VIII. Similar observations have also been reported for these spinal regions in cat (Carstens and Trevino,1978a) and monkey (Hayes and Rustioni,1980; also see Foreman et al.,1981).

Although double-labeled STT neurons were observed in all four groups of animals, the highest percentages were seen in the enlargements of the M-STT/MRF group (27% compared to 10 to 20% for other groups). These data are in general agreement with the double-label study of Kevetter and Willis (1983) in the rat. In concert with previous data from these laminae, the present results suggest that the STT neuronal population in this region project more often to the medial thalamus and that these STT neurons often issue axon collaterals to the MRF, but also to the PAG.

Laminae VII-VIII: Upper Cervical Segments

In contrast to more caudal segments, many STT neurons were found bilaterally in laminae VII and VIII in the upper cervical spinal cord. The distributions of both L-STT and M-STT neurons exhibited an ipsilateral predominance. So many STT neurons were labeled in these laminae that, together with the labeling in other layers, the majority of all STT neurons labeled were located in upper cervical segments. Such a pattern of labeling has been unanimously reported in those studies of the STT in

which data from upper cervical segments was shown (Carstens and Trevino,1978a; Giesler et al.,1979a; Hayes and Rustioni,1980; Kevetter and Willis,1983; Granum,1986; Kemplay and Webster,1986). Both L-STT and M-STT neurons were found to be double-labeled in high percentages, especially in the lateral ventral horns, from the MRF or PAG (30-40% of STT neurons). Thus, the double-label data are similar to those of Kevetter and Willis' study (1983) of STT axon collaterals to the MRF. In addition, the present study found a number of triple-labeled STT neurons in this region in all three cases. These present results, then, indicate that the overlap of populations of spinal projection neurons in the upper cervical spinal cord is unparalleled in other spinal segments. The distribution of labeled cells of all types is also unique and consistent with the upper cervical segments being a transition zone between the ventral spinal gray matter and the reticular formation of the medulla.

In a study focusing upon the ipsilaterally projecting STT neurons of the second cervical segment in cats, Carstens and Trevino (1978b) found neurons exhibiting physiological response properties which indicated that they received convergent input from neurons located in more caudal spinal segments. For example, some of these cells had large receptive fields and were activated by a variety of stimuli including noxious stimuli. Lesions of the caudal, ipsilateral, ventral or lateral funiculi often modified the receptive field characteristics of these

orthodromic responses, while stimulation of the dorsal columns sometimes inhibited the responses. In a few neurons, the thalamic stimulation that elicited an antidromic response also resulted in a delayed orthodromic response, suggesting that some of the afferent input to these neurons was from axon branches of other, more caudal, STT cells.

An unusual group of neurons were also identified by these authors. They were unresponsive to any type of peripheral stimulation. The authors speculated that these cells might be similar to unresponsive reticular formation neurons which can be antidromically activated from the spinal cord. Although extremely tenuous, such a suggestion is interesting since many neurons with similar morphology and in the same locations as those labeled from the ipsilateral thalamus can be retrogradely labeled from HRP injections into lower levels of the spinal cord (Yezierski et al.,1980; Leong et al.,1984; D. Newman, unpublished data). Taken together, these observations strongly suggest that the organization of the ventral gray matter in the upper cervical segments exhibits both spinal and reticular characteristics and, thus, presents a number of intriguing questions for future research.

Lamina VI and the Internal Basilar Nucleus of Upper Cervical Spinal Segments

The present data demonstrated clear differences between upper cervical lamina VI and the internal basilar nucleus. Lamina VI contained a moderate number of L-STT and M-STT neurons. Furthermore, some of these neurons were double-labeled, especially from the MRF (26% of L-STTs, 18% of M-STTs). In contrast, internal basilar neurons were heavily labeled following lateral thalamic injections, particularly those in which the injections impinged upon the posterior nucleus. These neurons were almost never double-labeled.

Since some previous studies did not discriminate between medially and laterally projecting STT neurons or between those with or without axon branches, the labeling in the internal basilar nucleus and lamina VI blended together to give the impression of a homogeneous cell group (Granum,1986; Kemplay and Webster,1986). Although the thalamic injections in Kevetter and Willis' study (1983) were large, dense labeling in the internal basilar nucleus was not reported. Nevertheless, other investigators have observed heavy labeling in the internal basilar nucleus following lateral, but not medial, thalamic injections (Carstens and Trevino,1978a&b; Giesler et al.,1979a). In addition, the present association of dense labeling in this nucleus with injections involving the posterior nucleus has also been made previously in the cat (Carstens and

Trevino,1978a&b).

As discussed in the spinomesencephalic section, the possibility exists that the internal basilar nucleus also projects to the caudal tectum. Therefore, future studies may find that L-STT neurons in this nucleus can be double-labeled from the tectum. The present data, however, demonstrate that additional projections from the internal basilar nucleus to the MRF or PAG are extremely sparse or nonexistent.

Lamina X

There were very few labeled STT neurons found in lamina X in the present study. When present, however, they were sometimes double-labeled from the PAG or the MRF. These data, then, do not conflict with any of the previous single- or double-label studies in rat (Giesler et al.,1979a; Kevetter and Willis,1983; Granum,1986; Kemplay and Webster,1986; Liu, 1986), cat (Carstens and Trevino,1978a), or monkey (Willis et al.,1979; Hayes and Rustioni,1980). Lamina X seems to be a region in which there is little overlap between a sparse STT population and the dense spinoreticular and spinoannular populations. Further, the sparse STT labeling is in sharp contrast to the heavy labeling observed following tracer injections into the hypothalamus (Burstein et al.,in press), underscoring the observation that these two tracts do not exhibit the same distribution.

Lateral Spinal Nucleus

Both L-STT and M-STT neurons were found in the lateral spinal nucleus at all spinal levels examined. There were generally more M-STT neurons labeled in the enlargements and these cells exhibited a bilateral distribution whereas the L-STT neurons were labeled mainly on the contralateral side. Both L-STT and M-STT neurons in the lateral spinal nucleus were double-labeled, but the distributions and percentages varied. In the lumbar enlargement and mid-thoracic segments, only a few double-labeled STT neurons were observed. In contrast, the cervical enlargement contained increased numbers of double-labeled neurons, especially in the L-STT/PAG and M-STT/PAG groups (up to 30% of STT neurons). Finally, there were double-labeled STT neurons in the upper cervical lateral spinal nucleus.

As discussed above, previous studies of ascending spinal systems have not always reported labeled cells in the lateral spinal nucleus. In studies of the STT, Giesler and his colleagues (1979a) did not report any of these neurons to be HRP-labeled from the thalamus. Kevetter and Willis (1983) reported that they observed labeled neurons in the nucleus, but these data were not quantified or illustrated in their paper. Later studies of Granum (1986) and Kemplay and Webster (1986) did illustrate labeled neurons in the lateral spinal nucleus. And, finally, Liu (1986) reported M-STT neurons, single- and double-labeled from the PAG, in

this area in the spinal enlargements. Differences in the tracers used may account for any apparent disagreement between the present findings and those of others in the rat. There are, however, species differences regarding this nucleus (Gwyn and Waldron,1969). The lateral spinal nucleus is not present in the cat. Although it is not known whether the nucleus exists in monkey, no neurons have been labeled in a similar location in retrograde studies of the STT in this species (Willis et al.,1979; Hayes and Rustioni,1980).

Many lateral spinal neurons have been labeled following tracer injections into the hypothalamus of rats (Burstein et al.,in press). Therefore, this nucleus, along with lamina V and lower cervical lamina I, are regions in which the cells of the spinohypothalamic tract may overlap with those of the tracts studied in the present work.

Lateral Cervical Nucleus

The labeling found in the lateral cervical nucleus in the present study was quite distinct and provides additional evidence for the concept that the lateral spinal nucleus is not a caudal extension of the lateral cervical nucleus (Giesler et al.,1979b; Giesler and Elde,1985), as originally proposed by Gwyn and Waldron (1968,1969). As observed in previous studies in rat (Giesler et al.,1979a) and cat (Carstens and Trevino,1978a; Berkley et al.,1980), many neurons were labeled in this nucleus in the present study following tracer injections into the lateral

thalamus. So few neurons were labeled in the nucleus following medial thalamic injections that these may well have been more properly placed in the adjacent lateral spinal nucleus since, as mentioned above, the boundaries between the two nuclei are not readily apparent. There were almost no double-labeled neurons in the lateral cervical nucleus, consistent with an absence of cells labeled from the PAG or MRF. However, since there is evidence that some cells in the lateral cervical nucleus project to the caudal tectum (Berkley et al.,1980; Menetrey et al.,1982; Flink et al.,1983; Wiberg and Blomqvist,1984), it is possible that some of these neurons may also issue axon collaterals to the tectum as has been shown by Berkley and her coworkers (1980) in the cat.

Ventromedial Dorsal Horn Group: Lumbar Enlargement

Only single-labeled L-STT neurons were found in the ventromedial dorsal horn of the upper lumbar enlargement in the present study. Heavy labeling in this area was associated with involvement of the ventral lateral nucleus in the thalamic injections. This group of neurons has been labeled consistently in previous studies in which large tracer injections were made into the thalamus of rats (Kevetter and Willis,1983; Granum,1986; Kemplay and Webster,1986). A similar group of cells has been labeled following HRP injections into the cerebellum of rats (Matsushita and Hosoya,1979). Although the present study

never found ventromedial dorsal horn neurons to be double-labeled, it might be possible that these neurons issue axon collaterals to the cerebellum.

The present data, however, seem to conflict with previous findings in the rat of comparable numbers of L-STT and M-STT neurons in the ventromedial dorsal horn (Giesler et al.,1979a). These authors noted that the ventromedial neurons were most densely labeled following large injections involving both medial and lateral thalamic nuclei. When their injections were restricted to either the intralaminar nuclei or to VPL, the density of HRP in labeled cells was diminished. Such an observation would be consistent with the possibility, suggested in the present study, that the ventromedial dorsal horn neurons project to the ventral lateral nucleus of the thalamus, since it is located between the intralaminar nuclei and the VPL in rats and cats (see Fig 3B). If this hypothesis is correct, then spread of tracer from either a medial or lateral thalamic injection could result in the labeling of ventromedial dorsal horn neurons. Unlike HRP, which cannot be visualized without chemical manipulation, fluorescent tracers can be seen directly at the injection sites. Thus, fluorescent tracers may allow for greater certainty in screening the locations of tracer injections. It is possible, therefore, that the only difference between the present data and those of Giesler et al. (1979a) is that, in the present work, there was less spread of tracer from medial thalamic injections

into the ventral lateral nucleus.

It is not clear whether the ventromedial dorsal horn group is present in monkey as it has not been apparent in the retrograde studies of Willis et al. (1979) and Hayes and Rustioni (1980). The STT terminals observed in anterograde studies in rat and cat in the caudal ventral lateral nucleus (Lund and Webster,1967b; Boivie,1971; Berkley,1983; Burton and Craig,1983) are also not seen in the monkey. Rather, STT terminals in the monkey are located in the oral parts of VPL and in the lateral posterior nucleus (Asanuma et al.,1983a&b; Burton and Craig,1983). Since these terminal regions in the monkey have connections similar to those of the ventral lateral nucleus of rat and cat, and represent an additional terminal field (separated from the classical terminations in VPL), it is possible that these terminations in the primate are homologous to those in the ventral lateral nucleus of rat and cat (see Burton and Craig,1983; and appropriate chapters in Jones,1985). If so, they were almost certainly missed in previous retrograde studies of the primate STT since the injection sites were understandably targeted to the caudal VPL, where the majority of primate STT fibers end (Mehler et al.,1960; Mehler,1969).

A densely packed group of cells similar to the ventromedial dorsal horn group has been observed in cat (Carstens and Trevino,1978a). This group was most apparent in cases with large thalamic injections, similar to the

findings of Giesler et al. (1979a). In addition, in the cat this group was located in a position slightly more ventral than that seen in the rat.

Admittedly, the correlations drawn between the data from the rat and those from the monkey are highly speculative. They are offered here mainly in the hope that future research may address the question of whether or not a similar cell group is present in primates.

In the context of possible STT projections to the ventral lateral nucleus which has connections with motor-related cortical areas, it is interesting that STT neurons in the ventromedial dorsal horn have been shown to respond to both cutaneous and proprioceptive input in the rat (Menetrey et al.,1984a). These authors found that ventromedial dorsal horn neurons were maximally excited by ankle extension but could be excited by cutaneous stimulation also. In addition, they were inhibited by noxious heat and their responses could be modified by ankle flexion. It was suggested by these authors that the STT neurons in the rat ventromedial dorsal horn function in the transmission of information important in locomotion. These data complement the present proposal that these cells project to the ventral lateral nucleus of the thalamus.

Physiological Response Properties of STT Neurons

Electrophysiological investigations of the STT have revealed that the response properties of STT neurons are, in many cases, the same as those of spinoreticular and spinomesencephalic tract neurons. However, many more studies have focused on the STT in comparison to the other two tracts and, thus, the information available about STT physiology is voluminous and detailed. Of particular relevance to the present data are only those studies which either distinguished between L-STT and M-STT neurons or those which reported specific types of response properties associated with STT neurons in particular spinal laminae. For additional information and references, the reader is referred to the review by Dubner and Bennett (1983) or to the summary contained in Willis' recent book (1985) on the peripheral and central mechanisms of pain transmission.

One of the earliest studies in which the response properties of antidromically activated STT neurons were characterized was that of Willis et al. (1974) in monkeys. While the activation sites in their study could not distinguish between L-STT and M-STT neurons, the laminar locations of activated units were noted. The cells' response properties generally corresponded to the three types discussed above: 1) wide-dynamic-range, 2) nociceptive-specific, and 3) deep types of responses. About 30% of the STT neurons in this study were nociceptive-specific units. They exhibited the lowest conduction

velocities (averaging 28.4 m/s) and were located in the dorsal horn, especially in lamina I and V. Wide-dynamic-range responses were found in 38% of the neurons, most of which were located in laminae IV and V. In contrast, the STT neurons which responded to deep stimulation of muscles and joints tended to be located in laminae VI-VIII.

Later studies in the rat found similar STT neuronal response properties (Giesler et al.,1976). Wide-dynamic-range neurons represented 37% of the sample and were located in the dorsal horn. In deeper laminae, STT neurons were found which responded to innocuous cutaneous stimuli or to stimulation of deep structures. These cells were in laminae VI-VIII and tended to exhibit complex receptive fields. Only a few nociceptive-specific neurons were found and these were located in lamina I.

An association of nociceptive-specific neurons to lamina I was strengthened by Craig and Kniffki (1985) for the cat and by the studies of Price et al. (1978) for the monkey. In the latter study, a high percentage of such neurons was located in lamina I. These authors also found many wide-dynamic-range neurons in lamina V and a few neurons responding vigorously to innocuous cutaneous stimuli in lamina IV. In addition, some STT neurons were encountered which could also be activated from stimulation sites in or near the PAG. Since all of the thalamic stimulation sites were in the VPL, these data of Price and his coworkers provided the first direct evidence that L-STT

neurons in the primate issue axon collaterals to the PAG.

By stimulating either medial or lateral thalamic regions in monkey, Giesler et al. (1981) were able to activate L-STT or M-STT neurons and, thus, assess their specific response properties and locations. The majority of L-STT neurons were encountered in the dorsal horn. Wide-dynamic-range neurons were most common in this group (68%), but nociceptive-specific (27%) and deep (5%) responses were also found. M-STT neurons were most common in the deeper laminae and the majority (62%) of these were nociceptive-specific while others were wide-dynamic-range (25%) or deep (12%) cells. Some neurons could be activated from both the medial and lateral thalamus (LM-STT). These LM-STT neurons exhibited response properties which mirrored those of L-STT neurons. Furthermore, Giesler and his coworkers found that some of these cells could also be activated from sites in the MRF.

There is evidence that the M-STT responses in cat differ from those in monkey. In a study which recorded the responses of M-STT neurons in laminae VII and VIII, wide-dynamic-range neurons comprised half of all the cells studied (Meyers and Snow,1982). Only 8% were nociceptive-specific. These data are in sharp contrast to those of Giesler et al. (1981) in the monkey, in which the majority of M-STT neurons were nociceptive-specific. Meyers and Snow also found that 30% of their sample responded to innocuous tactile stimulation while 11% did not respond to any type of

peripheral stimulation. These authors did agree with Giesler et al. (1981) in that both studies found the receptive fields of M-STT neurons in laminae VII and VIII to be large and complex, indicating a wide convergence of afferent input. It is interesting that responses similar to those described by Meyers and Snow (1982) have been reported in spinoreticular and spinomesencephalic neurons located in ventral laminae in cat and rat (Fields et al.,1977; Maunz et al.,1978; Menetrey et al.,1980; Yezierski and Schwartz,1986).

All of the studies discussed above have characterized the response properties of STT neurons in the lumbar enlargement. Although there is little information, the responses of STT neurons in the cervical enlargement are probably similar to those in the lumbar segments (Dilly et al.,1968). In thoracic segments, response properties identical to those in the lumbar enlargement have been demonstrated in monkey (Foreman and Weber, 1980; Foreman et al.,1981). Interestingly, Foreman and his colleagues have also found that there is convergent input to thoracic STT neurons from peripheral and visceral receptors. Such convergent input, which probably underlies the phenomenon of referred pain, has also been demonstrated for lower lumbar and sacral STT neurons (Milne et al.,1981).

As discussed in the section regarding the ventral laminae of the upper cervical spinal cord, there is evidence that the STT neurons in this region exhibit certain response

properties not typical of those observed at lumbar levels (Carstens and Trevino,1978b). There are, however, similarities between the responses of lumbar STT neurons and those of the cervicothalamic tract neurons in the lateral cervical nucleus. Both nociceptive-specific and wide-dynamic-range neurons have been found in the lateral cervical nucleus of rats and cats (Giesler et al.,1979b; Kajander and Giesler, in press). Some other neurons in this nucleus were found to respond solely to innocuous tactile stimulation. In cats, the lateral cervical neurons which respond to nociceptive stimulation are intensely sensitive to anesthetics and, thus, were not found in many previous studies (see Kajander and Giesler for discussion of this point).

The present findings of L-STT and M-STT neurons which issue axon collaterals to the PAG and/or MRF lead to the expectation that these cells' response properties should conform to those which have been demonstrated in electrophysiological studies of all three tracts. It is, therefore, necessary to briefly summarize those response properties which these tracts have in common. Such an endeavor is greatly complicated by the paucity of physiological data regarding the spinoreticular and spinomesencephalic tracts and by the differences in species, techniques and issues of focus in the studies which have been accomplished. Nevertheless, it is possible to summarize certain general associations based upon the

physiological studies which have been discussed above.

Wide-dynamic-range neurons have been found in studies of all three tracts. Lamina V and, to a lesser degree, laminae VII and VIII have been observed to contain STT, spinoreticular and spinomesencephalic neurons with wide-dynamic-range responses. The present study found more L-STT than M-STT neurons in lamina V; these were double-labeled from the PAG, MRF, or, sometimes, labeled from both regions. STT neurons in laminae VII and VIII were also double-labeled, especially from the MRF, and triple-labeled. Thus, there seems to be a considerable overlap between these tracts which can allow for the transmission of wide-dynamic-range type information to the thalamus, PAG and MRF.

In contrast, nociceptive-specific cells can only be associated unequivocally with the STT and spinomesencephalic tracts. These cells are common in lamina I but are occasionally found in lamina V. Although there is evidence that the proportion of nociceptive-specific to wide-dynamic-range neurons in lamina I differs across species, nociceptive-specific types are still the most numerous. Thus, it is interesting that in the present study lamina I contained mostly L-STT neurons which were double-labeled only from the PAG. Therefore, there may be a tendency for nociceptive-specific input from laminae I to be preferentially transmitted to the lateral thalamus and PAG.

Neurons responding to deep stimulation of muscles

and joints are plentiful in studies of the spinoreticular tract and present, but less common, in studies of the STT and spinomesencephalic tracts. Most neurons of this response type have been encountered in the ventral laminae (VII and VIII). The present study found STT neurons in this region, many of which were M-STT cells, to be double-labeled most frequently from the MRF, but also from the PAG. So, while deep stimulation seems to affect many more spinoreticular neurons, there is still a great deal of overlap between these neurons and those of the other two tracts. Each tract is capable of transmitting some information regarding stimulation of muscles and joints.

In summary, although these general features have been greatly simplified, there is a considerable overlap in the functional characteristics of the three spinal projection systems. The present findings of L-STT and M-STT neurons which issue axon collaterals to the PAG and MRF are consistent with and supported by the commonalities in physiological response properties observed in the STT, spinoreticular and spinomesencephalic tracts.

CONCLUSIONS

In considering the overlap between the anatomical and physiological data now available regarding the STT and other tracts of various mammalian species, it is appropriate to be more impressed, as was Mehler (1969), with the similarities than with the dissimilarities in the

organization of the somatosensory spinal systems. The results of the present study do not support the classical two-component concept of the STT which grew from interpretations of Mehler's work. For example, the two-component concept held that the neospinothalamic tract was a direct pathway to the lateral thalamus that lacked significant collateralization and was most prominent in primates. In contrast, the medially projecting paleospinothalamic tract was held to be composed of neurons which projected to the medial thalamus, but also issued axon branches to other brainstem regions. These regions, in turn, formed multi-neuron chains which eventually terminated in the medial thalamus. The present results have demonstrated that both L-STT and M-STT neurons issue axon collaterals to the PAG and MRF. Furthermore, the degree of collateralization was generally the same for both populations since, in any specific spinal region, the proportions of double- to single-labeled L-STT and M-STT neurons were comparable. Although exclusively single-labeled L-STT neurons were found in three regions, there is evidence from several species that two of these, the lateral cervical and internal basilar nuclei, may also project to the caudal tectum.

The present data offer no direct evidence to refute the idea that the laterally projecting STT becomes more prominent in higher mammals. However, comparison of previous retrograde labeling studies of the STT of monkey

yields very few differences between the organization observed in primates and that observed in the present study in rat. While the rat exhibits cell groups like the ventromedial dorsal horn which directly project to the lateral thalamus, it is not known whether all of these groups have a homologue in the primate. Only the projections from the lateral cervical nucleus to the lateral thalamus are clearly similar in rat and monkey. In addition, in the present study, the total number of labeled L-STT neurons was greater than the number of labeled M-STT neurons indicating that the L-STT is the major projection to the thalamus from the spinal cord even in the rat.

It is important to remember that the increase of laterally projecting fibers observed in primates was based on degeneration studies (Mehler,1957,1969). The physiological studies cited above have shown large differences in the average conduction velocities of rat versus monkey STT neurons (14-26 m/s and 40.3 m/s, respectively; Giesler et al.,1976; Willis et al.,1974). These differences suggest that STT axons in the rat are of smaller caliber than those of monkey and, thus the cell bodies themselves are smaller. Since degeneration techniques tend to stain larger, more heavily myelinated axons, the difference between monkey and rat seen by Mehler could well have been more apparent than real.

A possible objection to this last point could be that Mehler also reported very little difference between

these species in the number of degenerated fibers in the MRF, PAG, and medial thalamic nuclei (Mehler,1957,1969). However, the present data and those of others cited above have shown that many of the cell bodies of origin for these projections tend to be located in ventral laminae, especially layers VII and VIII. These cells tend to be larger than those in the dorsal horn, which contains the majority of L-STT neurons, and their axons are probably larger than those of dorsal horn neurons. Thus, the axons of ventral horn neurons in both species would be the most likely to be visualized with degeneration techniques.

The concept which emerges from recent research (including the present study) is that the STT is a multi-component system which serves to transmit a myriad of somatic and visceral sensations to the thalamus. These sensations include touch, deep pressure, temperature, and pain caused by mechanical, thermal and chemical stimuli. The present findings indicate that much of this information gains access to both specific and non-specific thalamic nuclei via the direct projections of L-STT and M-STT neurons. In addition, this information is transmitted to the MRF and PAG by the direct projections of spinoreticular and spinoannular neurons and by the collateral projections of both L-STT and M-STT neurons.

Interpretation of the present data in terms of electrophysiological investigations of the STT and other tracts suggests that there is rarely a clear association

between a particular type of sensation and a specific group of spinal projection neurons. Only the lumbar ventromedial dorsal horn neurons seem to exhibit unusual and homogeneous response properties relevant to locomotion. In addition, there are a large number of L-STT and spinomesencephalic neurons (some of which are the same cells) in lamina I of the dorsal horn and many of these are nociceptive-specific neurons.

In view of the variety in function and connectivity among STT neurons, it is probably not justified to conceive of L-STT neurons as mediating the sensory-discriminative aspects of pain perception or of M-STT neurons as mediating the emotional and motivational components of pain, as was suggested by Melzack and Casey (1968). Although their theory was based on the important differences in the peripherally evoked responses of medial versus lateral thalamic neurons, they did incorporate the two-component model of the STT into their description. The different functional aspects of pain should remain, however, as constructs applicable only to thalamic nuclei and not to STT neurons.

It is useful to consider that, although L-STT neurons outnumbered M-STT neurons in the spinal cord, the medial thalamus receives a much wider variety of inputs indirectly from the spinal cord than does the lateral thalamus (especially the VPL). In addition to the STT projection, the medial thalamus receives input from the MRF

and PAG, regions which the present study has shown to receive a variety of afferent projections including axon branches of L-STT neurons. Brainstem areas such as the parabrachial region, tectum, and pontine and mesencephalic reticular formation also receive spinal input and, in turn, provide afferents to the medial thalamus (see Jones,1985). Thus, the medial thalamus receives an immensely convergent input from widespread sources. Since the medial thalamus itself projects to wide areas of the cerebral cortex, it still can be considered to be a likely center for mediating the emotional and motivational aspects of somatosensory perception.

In contrast, the VPL, which receives much of the L-STT projection, receives essentially only two additional inputs originating in the spinal cord. One of these inputs originates from the dorsal column nuclei which relay somatotopically precise tactile information to the VPL. The other input comes from the spinocervicothalamic tract through projections of the lateral cervical nucleus to VPL. The efferent projections of the VPL are to the primary somatosensory cortex, thus placing the VPL in a unique position to mediate the sensory-discriminative components of somatosensory experience. Therefore, it is not surprising that the responses of VPL neurons to peripheral stimulation exhibit a somatotopic organization and modality specificity which neurons in the medial thalamus do not, forming the basis of Melzack and Casey's theory (1968). These different

physiological responses cannot be explained by clear differences in the response properties of L-STT versus M-STT neurons since these are often similar. It is, rather, the differences in the level of convergence and interactions of inputs from other sources which probably determine the wide functional separation of the medial versus lateral thalamus.

The findings that L-STT and M-STT neurons provide axon collaterals to the MRF and PAG have implications in terms of the descending systems which are proposed to modulate spinal activity. Electrical stimulation of both these regions results in an analgesia which is due to the inhibition of dorsal horn neurons by cells in the nucleus raphe magnus (Basbaum and Fields,1978,1984). Thus, the axon branches of STT neurons to these regions may function in the activation of these descending, inhibitory pathways. Of particular interest in this context are the lamina I nociceptive-specific neurons which project to the PAG, lateral thalamus or both.

There are many questions regarding the anatomy and physiology of the STT which remain unanswered. Further investigation may be able to more clearly define specific functions of particular types of STT neurons such as those in lamina I with collateral branches to the PAG. The interactions of the STT with other systems such as the spinohypothalamic tract also warrant examination in a variety of mammalian species. In addition, there is nothing known about the physiology of the internal basilar nucleus

which provides a significant input to the lateral thalamus and, probably, the posterior nucleus. Cell groups such as the ipsilaterally projecting ventral horn neurons in the upper cervical spinal cord provide intriguing questions concerning propriospinal connections and possible dual ascending and descending projections. In general, there is little information regarding the physiology of cells in the more ventral laminae which contribute fibers to the STT, spinoreticular and spinomesencephalic tracts.

Despite almost a hundred years of intense research, there is still much to be gained from study of the mammalian STT. As the multi-component nature of the STT becomes recognized, the challenges inherent in this area of study become greater, as do the rewards.

APPENDIX

LIST OF ABBREVIATIONS

AD	anterodorsal thalamic nucleus
AV	anteroventral thalamic nucleus
cc	corpus callosum
CL	central lateral thalamic nucleus (centrolateral)
CM	central medial thalamic nucleus
DY	diamidino yellow dihydrochloride
eml	external medullary lamina
f	fornix
FB	fast blue
FG	fluoro-gold
G	gelatinosus nucleus of thalamus
Hb	habenular nuclei
HC	hippocampal formation
HRP	horseradish peroxidase
IBN	internal basilar nucleus
ic	internal capsule
icp	inferior cerebellar peduncle
iml	internal medullary lamina
Lat VH	lateral ventral horn
LCN	lateral cervical nucleus
LD	laterodorsal thalamic nucleus
LP	lateral posterior thalamic nucleus
LSN	lateral spinal nucleus
L-STT	spinothalamic neurons labeled from the lateral thalamic injections

MD	mediodorsal thalamic nucleus
mlf	medial longitudinal fasciculus
MRF	medullary reticular formation
M-STT	spinothalamic neurons labeled from the medial thalamic injections
MVe	medial vestibular nucleus
PAG	periaqueductal gray
PC	paracentral thalamic nucleus
Po	posterior thalamic nuclear group
PrH	prepositus hypoglossal nucleus
PVA	anterior paraventricular thalamic nucleus
pyr	pyramidal tract
RaM	nucleus raphe magnus
Re	reuniens thalamic nucleus
RGc	nucleus reticularis gigantocellularis
RhS	rhodamine-labeled latex microspheres
RMc	nucleus reticularis magnocellularis
RPgcd	nucleus reticularis paragigantocellularis dorsalis
Rt	reticular thalamic nucleus
SAT	spinoannular tract
sm	stria medullaris thalamus
SO	superior olivary complex
SpC	spinal extension of nucleus cuneatus
SpG	spinal extension of nucleus gracilis
Sp5	spinal trigeminal nucleus
sp5	tract of the spinal trigeminal nucleus
SRT	spinoreticular tract

STT	spinothalamic tract
vhc	ventral hippocampal commissure
VL	ventral lateral thalamic nucleus (ventrolateral)
VM	ventromedial thalamic nucleus
VMDH	ventromedial dorsal horn
VPL	ventral posterolateral thalamic nucleus
VPM	ventral posteromedial thalamic nucleus
ZI	zona incerta
7	nucleus of 7th nerve (facial nucleus)
7n	7th nerve (facial nerve)

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